PROCEEDINGS.

VOL. XXVII. APRIL, 1930. No. 7.

Missouri Section.

St. Louis University School of Medicine, March 12, 1930.

4882

Some Observations on Circulatory Changes in a Renal Glomerulus.

H. L. WHITE.

From the Department of Physiology, Washington University School of Medicine.

In a female Necturus, anesthetized with urethane, a glomerulus showing sluggish blood flow was observed; the capillary loops were dilated, although not uniformly. The picture was unchanged by pithing the brain. This glomerulus was peculiar in that both afferent and efferent vessels were clearly seen; the afferent vessel came direct from a large ovarian artery. There was no alternation of flow. The afferent vessel was now stroked gently with a fine glass rod; there was no response. More vigorous stroking resulted in a shifting of the kidney region in the field after about a 5 second latent period; this was followed in 5 seconds more by constriction of the afferent vessel to the point of cessation, complete or practically so, of cell movement in the glomerulus. The shifting of the kidney region preceding afferent vessel constriction was seen to be due to a contraction of the ovarian artery from which the afferent vessel sprang, resulting in a tugging on the kidney. It was then found that traction on the ovarian artery was an adequate stimulus to this vasoconstriction, which spread from the artery to the afferent vessel. After this experiment had been repeated a few times at intervals of 3 or 4 minutes the phenomenon would occur without external stimulation; previous to its first production the flow had been steady for the 20 minute period of observation.

Both between and during periods of afferent vessel constriction the efferent vessel was seen to be constantly constricted, being narrower than the narrowest loop in the capillary tuft. Cells passed through it rapidly and in single file. Actual observation of such a constricted efferent vessel strengthens an idea which has been held for some time, that the slow blood flow through dilated glomerular capillaries at a time when the general circulation is vigorous is due to efferent vessel spasm.

The series of changes during a period as noted above was as follows. At first after mechanical stimulation and later spontaneously, at intervals of 4 or 5 minutes, the first step was a slowing, then cessation, then reversal of flow in the ovarian artery. This was due to a constriction which obviously involved the artery itself and probably its terminations; the reversal of flow was apparently due to the continued constriction of the artery after its peripheral branches were blocked, this constriction eventually involving the afferent vessel. The artery was about 400μ wide normally, about 280μ during constriction. The flow of blood in the accompanying ovarian vein was slowed but did not stop; the circulation in the adjoining glomerulus on either side of the one under observation was not affected. A few seconds after the first change was noted in the ovarian artery flow the afferent vessel disappeared from view and glomerular flow suddenly stopped, apparently in all the loops simultaneously. The capillary tuft appeared to collapse toward the arterial pole; the afferent vessel was emptied of cells but not all of the capillary loops were. The point of greatest interest is the appearance of the efferent vessel during such a cycle. Its diameter was apparently unchanged; usually an altered flow persisted in the efferent vessel through an entire cycle, at other times, when the afferent vessel constriction presumably was more vigorous, it stopped entirely. It was evident that the ratio of plasma to cells in the blood traversing the efferent vessel during periods of afferent constriction was greater than between such periods. These observations lend support to the idea expressed by Richards and Schmidt[1] that plasma skimming may occur in the afferent vessel. The mere fact that some forward movement of cells may be seen in the glomerular loops for some seconds after the afferent vessel has disappeared from view is open to two interpretations, plasma skimming in the afferent vessel or independent contractility of the loops. The behavior of the flow observed in the efferent vessel indicates that the former interpretation is correct. For if the slow and occasional

[1] Richards, A. N., and Schmidt, C. F., *Am. J. Physiol.*, 1924, lxxi, 178.

movement of cells seen in the capillaries during such a period were due only to capillary contractions, the afferent vessel being completely closed, it seems unlikely that the passage of these cells through the efferent vessel would be as rapid as was observed during this time. The increased ratio of plasma to cells must mean that the amount of plasma traversing the efferent vessel was more than could be accounted for by the mere squeezing out of blood preexisting in the capillaries. The fact that not all of the loops were emptied of cells at a time when there was, as evidenced by the appearance of the efferent vessel, a fairly rapid passage of plasma through the glomerulus may mean that the passage was largely confined to those loops which were emptied or that the diminished flow was insufficient to overcome in all cases the adhesion of the cells to the capillary walls. The assumption of a constriction at the junction of capillaries with efferent vessel would hardly help to explain this finding; in that event the cells should have been piled up at the efferent ends of the capillaries, which was not the case.

It has seemed worth while for several reasons to report these observations made on a single glomerulus. In the first place this is the first report so far as I know, of observations on a glomerulus whose efferent vessel was visualized during cessation of cell movement in the afferent vessel. At the time of the observations no one had reported having seen both afferent and efferent vessels of the same glomerulus in the living kidney. Only a few days later a paper by Bieter[2] appeared in which he mentions seeing both vessels in frogs' glomeruli but gives no description of the efferent vessel during afferent constriction. The significance of the efferent vessel picture in attempting to decide the question of capillary contractility has already been referred to. In the second place actual visualization of a constricted efferent vessel with a slow blood flow through dilated capillary loops affords an explanation for a type of glomerular flow often seen in Necturus. It was only natural to assume this explanation but until an efferent vessel could be seen under these conditions it could be only an assumption. In the third place the origin of an afferent vessel directly from a large ovarian artery is interesting, although probably not common. This vessel was seen throughout its entire length; it was 240μ long and, between periods of constriction, about 40μ wide. This peculiar origin of the afferent vessel, if it could be shown to occur with any significant frequency, which it probably does not, might afford an interesting explanation of a urine flow in the amphibian kidney after ligation of all the

[2] Bieter, R. N., *Am. J. Physiol.*, 1930, xci, 436.

renal arteries. The first part of the efferent vessel could not be seen, as it lay dorsal to the glomerulus. It could be seen, apparently just dorsal to the dorsal wall of the capsule, through the clear space that separated the capillary tuft from the capsule wall. The segment thus seen was about 150μ long and was narrower than the narrowest capillary in the glomerulus, being not more than 20μ wide. It could not be followed beyond the edge of the capsule. The fact that both vessels could be seen in this glomerulus is almost certainly due to the atypical arrangement.

It will be noted that the events described here are probably not the same as the alternation of glomerular flow described by Richards and Schmidt and by Bieter. In their experiments on the frog alternation of glomerular flow was a frequent spontaneous occurrence and there was no reason to believe that the vascular constriction involved anything central to the afferent vessel. In the observations reported here the afferent vessel constriction was secondary to that of the large artery from which it sprang; the phenomenon did not occur until initiated by mechanical stimulation, although after having been so initiated it recurred rhythmically.

4883

Use of the Interferometer for Serum Protein and Protein Fraction Determinations.

WILLIAM J. DIECKMANN. (Introduced by P. A. Shaffer.)

From the Department of Obstetrics and Gynecology of the Washington University School of Medicine.

Adams[1] stated that the interferometer was a precision instrument suitable for all sorts of determinations in which the refractometer has been used, with the additional advantage of being much less susceptible to temperature change and much more accurate. He states that with the refractometer one must regulate the change in temperature to 0.01° in order to secure an accuracy of one unit in the sixth place, but the interferometer requires no special regulation of temperature to secure an accuracy of one unit in the seventh place. In determining serum proteins, we found that a serum which read 454 at 15°, would read 452 at 30°, the difference being equivalent to 0.014% protein. Therefore, all readings have been made at room temperature.

[1] Adams, L. H., *J. Am. Chem. Soc.*, 1915, xxxvii, 1181.

The water interferometer is supplied with 5, 10, 20, and 40 mm. chambers. The 5 and 10 mm. chambers can be fitted with a 4 and 9 mm. insert, respectively, thus furnishing a chamber 1 mm. in depth with the advantage that it can be easily cleaned on removal of the insert. The scale of the instrument is divided into 3000 divisions or Trommelteil (T.T.), but we limit our readings to 1500 T.T., because it has been found that if the concentration of a solution is plotted against the T.T., the line becomes curved above this figure.

All interferometer chambers have to be standardized against known solutions. We used the method described by Robertson for preparing standard protein solutions, and found that a 1% solution of serum protein gave a reading in the 1 mm. chamber of 136.

Hirsch[2] described a method for determining serum proteins, and the method used by us is similar with the exception that we do not dilute the serum. The reading is obtained of the serum and also of the filtrate (electrolyte, urea, etc.), which has been obtained by heating one volume of serum and one volume of 0.04 normal acetic acid in a sealed tube. The difference between the two readings represents the coagulable protein and the percentage is derived by dividing the T.T. by 136. Serum protein can be determined quickly, accurately, and easily on 0.75 cc. serum. Furthermore, one can determine concentrations of protein of less than 0.1% in the 5 mm. chamber and of still greater dilution in the larger chamber.

Fibrin was obtained from 1 cc. of plasma, using Wu's method, and dissolved in 2 cc. of 0.05 normal NaOH. The solution was read in the 10 mm. chamber, and the difference between the fibrin reading and that of the NaOH, divided by 1090 (1% protein in the 10 mm. chamber), multiplied by 2, gives the percentage of fibrin in the sample.

The albumin globulin ratio was determined by precipitating the globulin from 1 cc. of serum by adding 1 cc. of a 44% solution of Na_2SO_4. The tube is stoppered, shaken, and allowed to stand in the incubator for 3 hours. It is filtered in the incubator and the filtrate is read against a standard similarly prepared with water and Na_2SO_4, using the same pipette that was used for the serum. The reading obtained multiplied by 2, is the interference produced by the albumin and non-protein elements. The difference between this figure and the reading of the serum is assumed to be globulin.

[2] Hirsch, P., *Handbuch der Biol. Arbeits.*, 1926, II, Part 2, Section 1, 737.

4884

Observations on the Contraction of Fibrin in Blood.

JOHN AUER.

From the Department of Pharmacology of St. Louis University School of Medicine.

The hanging-drop method was employed exclusively, with aseptic precautions. The blood preparation was sealed with sterile vaseline. The optical equipment consisted of a Zeiss apochromat microscope; objective 3 mm. 1.4 N.A. oil immersion gave the best results, though the 2 mm. 1.3 N.A. is satisfactory. The light source was a Zeiss filament lamp. The blood was taken from the human subject, dogs, rabbits, and guinea pigs; the results noted in the different animal species are practically the same; those reported here are based on approximately 100 different preparations. Each observation lasted between one and 6 hours. The observations were made at room temperature; the coagulation time varied in the different bloods; those clotting with moderate rapidity were most suitable for study.

Observation of the edges of the blood-drop sooner or later shows in some area a distortion of the oval or round red corpuscles by fibrin threads which have anchored themselves to the periphery of the corpuscle. These fibrin filaments generally stretch at right angles to the periphery of the drop and appear attached to the serum edge of the drop (often to a blood-shadow) and to the main body of the blood-drop itself. Commonly 2 fibrin filaments are cemented to opposite sides of a corpuscle and the corpuscle then assumes a spindleshape due to contractin of the fibrin filaments. The fibrin threads may appear short at first, 5μ and less, and may seem to end in the clear serum. Gradually more of the fibrin filament becomes visible, at times showing a length of 40μ; the section adjacent to the red cell thickens somewhat in diameter, becoming occasionally slightly fuzzy in outline and this same portion may show a faint diagonal striation. This diagonal striation may be that of a right screw thread or a left, or it may change from one to the other during observation. These fibrin threads shorten in length and the attached red corpuscle becomes slenderer and more spindle-shaped. Suddenly one of the anchoring fibers snaps at the peripheral end and the red corpuscle darts across a section of the field as a pear-shaped body. The severed fiber may roll up into a glistening, refractile nodule which adheres to the round side of the red corpuscle while the other fibrin thread now shows more or less active motion; it sways, rotates on its short

axis, twists, forms large open loops, straightens out, etc. (Fig. 1.)

Instead of forming a nodule, the torn fibrin filament at times shortens and thickens moderately, sways stiffly, rotates, shows diagonal striations either right or left or changing from one to the other and then abruptly a series of equidistant, refractile beads appear one after the other in the thickened thread, beginning at the periphery. This process may occur in steps or be completed abruptly, but finally a chain of refractile beads writhes, twists and rotates actively. (Fig. 1.) The tip bead describes circles, ovals and apparently figures-of-eight when in the optical axis; it moves in all planes approaching the point of fixation in loops and then straightening out. Occasionally the terminal bead is twisted off and then it rolls and quivers free in the serum, indistinguishable from so-called blood-dust. Or the entire chain of beads breaks away and now rolls, trembles and sways away in the serum.

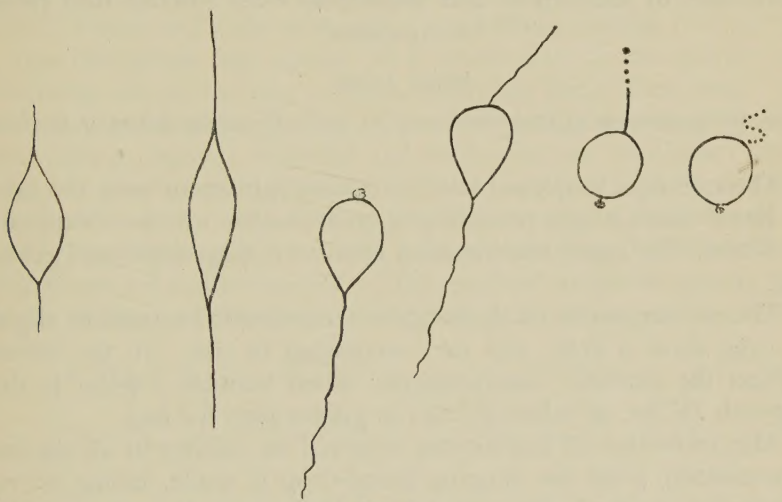


FIG. 1.

Some of the changes observed in fibrin-threads anchoring a red corpuscle in normal clotting blood. Sketches are a composite of two cells.

The refractile nodule formed on a red corpuscle by the contraction of a fibrin thread slowly disappears; whether it is dissolved on the surface of the red corpuscle or by the serum, is impossible to state; on the blood-shadows the refractile globules may persist for hours.

It was noted several times that a severed contracted fibrin filament moved across the surface of a red corpuscle and then moved back, or that a microcyte adherent to a red cell at the point of attachment of an actively vibrating fibrin flagellum, was slowly moved continuously

in one direction and then slowly in a directly opposite direction. The explanation for this will be given in a subsequent paper.

Polarized light with compensator (Red, first order) gave no definite evidence that the refractile nodules and beads were anisotropic.

More than 2 fibrin filaments may anchor a red corpuscle; many times a red corpuscle more or less crenated may be seen with 3 and even 4 actively vibrating, rotating and even lashing fibrin flagella. The action of these flagella or even of a single one imparts a tremulous quiver to the red corpuscle which undoubtedly heretofore has been interpreted as Brownian movement.

4885

Formation of Macrocytes and Microcytes from Normal Red Blood Corpuscles.

JOHN AUER.

From the Department of Pharmacology of St. Louis University School of Medicine.

The technique employed and the optical equipment were the same as those noted in my preceding communication on the contraction of fibrin.[1] The blood was obtained from man, dog, rabbit and guinea pig.

The red corpuscles of all the species examined are round or slightly oval, show a della, and vary somewhat in size. In the human subject the diameter measurements varied between 7.8-9μ; in dog between 7-7.8μ; in rabbit 6-7.8μ; in guinea pigs 6-7.8μ.

The formation of macrocytes occurred as follows in all species: Immediately after the hanging blood-drop is made, laking of red corpuscles begins in the periphery. If 2 corpuscles are touching each other, the area of contact slowly becomes greater until the 2 reds form an oval mass with an encircling median groove. In the middle of this groove a whitish line is seen which represents the opposed or fused surfaces of the 2 corpuscles. When this common septum disappears the 2 corpuscles form a round or slightly oval macrocyte measuring 10μ+ in diameter. As long as the septum exists the macrocyte shows a slight indentation in its outline. (See Fig. 1.) The hemoglobin content is usually the same as that of a normal red corpuscle, which shows that the thickness of the macrocyte is not appreciably greater than that of a normal erythrocyte.

[1] Auer, John, PROC. SOC. EXP. BIOL. AND MED., 1930, xxvii, 618.

No della was ever observed in a macrocyte, nor was this structure seen in sideview.

Macrocytes may also be formed by the fusion of 3 corpuscles or by fusion of a macrocyte with a normal corpuscle. Fusion of component red corpuscles is not always complete; this is readily betrayed by the presence of septa and by the shape.

After formation, the macrocytes sooner or later begin to pale without obvious change in size and finally a macrocytic blood-shadow larger than that of the average blood-shadow is formed. The whole process varies in duration from 10 to 30+ minutes.

Microcytes were observed in the process of formation in all the species of blood examined. There are two general methods: (a) A red corpuscle has been changed to a spindle-shaped structure by the shortening of 2 anchoring fibrin-threads. This spindle cell is stretched and relaxed repeatedly by a shortening of the anchoring fibrin threads and their subsequent lengthening; during this process a few crenations may appear on a small area of the spindle red. Suddenly one or the other of the anchoring fibrin-fibers snaps and the red darts across a portion of the field changing during observation into a coarsely crenated red corpuscle, the crenations being broad at the base and blunt at their tip; attached to this crenated corpuscle one now sees a long vibrating, rotating flagellum and on another part a refractile nodular mass representing the fibrin-thread which tore off and contracted. This crenated corpuscle quivers, due to the motility of the attached flagellum, and the crenations slowly

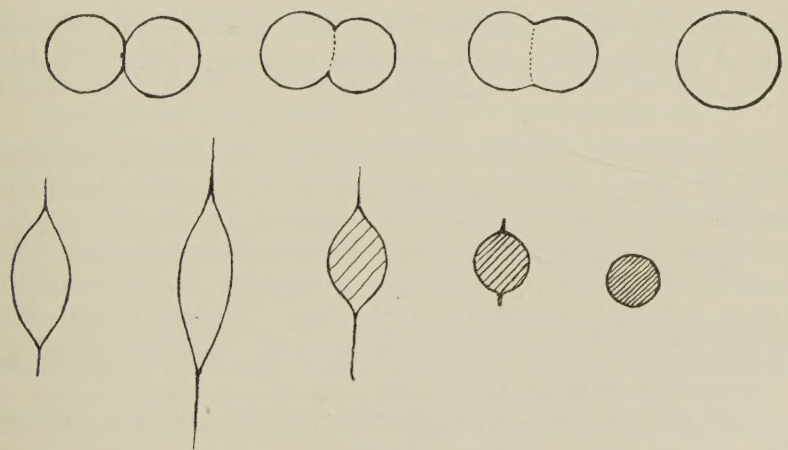


FIG. 1.

(a) Formation of a macrocyte. Sketches represent the same cells throughout.
(b) Formation of a microcyte. Sketches represent changes observed in the same cell.

change, becoming narrower at the base and more pointed. Within a shorter or longer period the crenated corpuscle becomes rounded and smaller, the crenations become thin and slender and the hemoglobin tint is definitely deeper than in the coarsely crenated stage. At this stage the refractile nodule has disappeared and the vibrating, twisting flagellum may or may not be present. Finally, the needle-like spicules disappear from the cell and a perfectly round, smooth microcyte 5μ in diameter without della and with dense hemoglobin has been formed.

(b) The other method observed is as follows: A spindle red corpuscle is observed slowly growing smaller, remaining apparently motionless during the process, while the anchoring fibrin-threads become more prominent and definitely thicker at their point of attachment to the corpuscle. The hemoglobin tint deepens, the cell becomes rounder and finally a typical microcyte with dense hemoglobin content, and without della appears. In this method the non-hemoglobin constituents have been wrung out of the corpuscle by the twisting of the anchoring fibrin threads which tear, contract and disappear at a certain time. See Fig. 1.

4886

Effect of Various Anterior Pituitary Preparations on Basal Metabolism in Guinea Pigs.

WALTER J. SIEBERT AND ROBERT S. SMITH. (Introduced by Leo Loeb.)

From the Department of Pathology, Washington University School of Medicine, St. Louis, Mo.

Loeb,[1] and later Loeb and Bassett,[2] and Silberberg[3] studied the effects of various anterior pituitary preparations upon the thyroid gland; the former investigators also correlated the changes in the thyroid thus produced with those that take place in the sex organs under the influence of anterior pituitary preparations.[4] It was found that various preparations have different effects. Intraperitoneal injections of acid and alkaline extracts of fresh cattle glands cause a

[1] Loeb, Leo, *J. Med. Res.*, 1920, xli, 481.

[2] Loeb, Leo, and Bassett, R. B., PROC. SOC. EXP. BIOL. AND MED., 1929, xxvi, 860.

[3] Silberberg, Martin, PROC. SOC. EXP. BIOL. AND MED., 1929, xxvi, 166.

[4] Loeb, Leo, and Bassett, R. B., PROC. SOC. EXP. BIOL. AND MED., 1930, xxvii, 490.

very marked hypertrophy of the thyroid gland in a very short time; while on the contrary the feeding of Armour's anterior pituitary tablets produced structural changes indicating a decreased activity of the gland. Loeb and Siebert[5] found that the oral administration of anterior pituitary substance prepared in our laboratory does not produce the same effects as the Armour preparation. According to Loeb and Bassett[2] repeated subcutaneous inoculations of anterior pituitary gland tissue obtained from freshly killed guinea pigs caused only very slight hypertrophic changes, if any, in the thyroid gland, while inoculations with somewhat larger quantities of fresh cattle gland produced definite hypertrophic changes.

In view of the effect of these preparations on the thyroid gland in guinea pigs and considering the significance of the thyroid gland in basal metabolism, we thought it of importance to study the action of these various anterior pituitary preparations on the basal metabolism in guinea pigs.

Our principal findings are as follows:

1. Daily subcutaneous injections of either acid or alkaline extracts of anterior pituitary glands of cattle cause a very marked and rapid rise in basal metabolism, which reaches a maximum of approximately +60% within the first 10 days. Then the basal metabolism gradually returns to a level approximately 15% higher than the average found in normal guinea pigs.

2. Feeding daily one 5 grain Armour anterior pituitary tablet to a guinea pig causes a gradual steady rise in basal metabolism which reaches a maximum of approximately +60% in about 30 days. There seems to be a cumulative effect in this case, and there is no tendency on the part of the metabolism to return to a lower level subsequently throughout the period of our investigation.

3. Feeding daily 5 grain pills of dried anterior pituitary substance prepared in our laboratory from fresh cattle glands causes a slight gradual rise in basal metabolism which reaches its maximum of approximately +25% in about 20 days, after which there is a gradual return to almost the normal level.

4. Daily subcutaneous inoculations of fresh guinea pig anterior pituitary gland tissue cause a very slight gradual rise in basal metabolism which reaches a maximum of approximately +15% in about 5 days, after which there is a decline. These latter experiments extended only over a period of 10 days, whereas in the former ones

[5] Loeb, Leo, and Siebert, W. J., PROC. SOC. EXP. BIOL. AND MED., 1930, xxvii, 495.

the effects of the above mentioned preparations were studied over periods of 30 days.

We may therefore conclude that all the various preparations of anterior pituitary substance which we used cause a rise in basal metabolism; however, the subcutaneous injection of acid or alkaline extracts produces the most rapid and greatest rise. Also the other preparations differ in the degree and the sharpness of the rise, and in the character of the curve following the period when they have reached the maximum point. The basal metabolism of all the animals, except those fed with Armour's anterior pituitary tablets, returns approximately to the normal level after some time, notwithstanding the continued administration of the various preparations during this period. These experiments furthermore suggest that some of these preparations may affect the basal metabolism, at least partly, through changes which they produce in the thyroid gland, while others affect it independently of such changes. We intend to study this question more directly in subsequent experiments.

4887

Induced Oxidations in Blood. Hemoglobin Destruction by Methylene Blue in Lactic Acid Peroxidation.

W. B. WENDEL. (Introduced by P. A. Shaffer.)

From the Laboratory of Biological Chemistry, Washington University School of Medicine.

We have previously reported experiments[1] which showed that the observations of Barron and Harrop[2, 3] that methylene blue added to blood increases the rate of oxygen consumption and decreases lactic acid production, may, in part, be accounted for by the oxidation of formed lactic acid. The incubation of washed dog erythrocytes in a solution containing added dl-lactate, in the presence but not in the absence of methylene blue, results in a disappearance of lactate.

Further study indicates that the lactic acid is oxidized to pyruvic acid, apparently quantitatively. That this oxidation is not mediated by the lactic "dehydrogenase" of the Wieland school is evi-

[1] Wendel, PROC. SOC. EXP. BIOL. AND MED., 1929, xxvi, 865.

[2] Harrop and Barron, *J. Exp. Med.*, 1928, xlviii, 207.

[3] Barron and Harrop, *J. Biol. Chem.*, 1928, lxxix, 65.

dent from the fact that molecular O_2 alone is incapable of effecting it; the dye is necessary. Experiments of Harned (unpublished) in this laboratory, indicating intermediate peroxide formation when leucomethylene blue undergoes oxidation, suggest that the mechanism by which the lactic acid is oxidized may be one of peroxidation. We find, however, that lactic acid is not affected by the hypothetical methylene blue peroxide alone, since shaking leucomethylene blue and buffered sodium lactate with O_2 fails to touch the lactate. Evidently another cell constituent is also essential.

A series of experiments with oxygenated dog erythrocytes (substantially free from sugar) in phosphate buffers containing added dl-lactate were carried out with the following determinations at intervals during 2 to 6 hours: O_2 content and capacity, CO_2 content, lactic acid and pyruvic acid. The following experiment illustrates the results obtained.

Experiment 128. Dog erythrocytes separated from serum and leucocytes by centrifugation, incubated 1.5 hours at 37°C, suspended in isotonic NaCl and phosphate buffer (pH 7.4) containing sodium dl-lactate and 0.005% methylene blue. Incubated with gentle shaking at 37°C. in closed filled tubes containing glass beads. Separate tubes analyzed at intervals. Results expressed in mMols per liter of mixture.

Hours:		0	2	4	6
Lactic acid.	Content	9.9	8.6	7.65	7.2
	Decrease	—	1.3	2.25	2.7
Pyruvic acid:	Content	0.7	2.5	3.7	4.6
	Increase	—	1.8	3.0	3.9
Oxygen:	Content	11.8	9.0	7.5	6.8
	Decrease	—	2.8	4.3	5.0
	Capacity	11.8	9.2	7.7	6.8
	—Decrease	—	2.6	4.1	5.0
O_2 required for oxidation of lactic to pyruvic acid:		—	0.65	1.12	1.35
O_2 required to oxidize hemoglobin to methemoglobin.		—	0.65	1.02	1.25
O_2 to other substances:		—	1.5	2.16	2.4

The results of these experiments appear to indicate a close relation between the hemoglobin destruction, as measured by loss of O_2 capacity and the oxidation of lactic acid to pyruvic acid. If it be supposed that hemoglobin be oxidized to methemoglobin, the O_2 required in all cases is approximately the same as required in the lactic acid oxidation. The sum of these fractions leaves at least one half of the O_2 for other oxidations. The CO_2 production varies, the total R.Q. of the process being 0.5 to 0.8.

Our tentative hypothesis of the mechanism of the process is as follows: Some reducing substance (inductor) present in the corpuscles is oxidized by methylene blue. The leucomethylene blue forms a peroxide-like compound with oxyhemoglobin. This complex peroxide effects the oxidation of lactic to pyruvic acid, and is itself thereby converted to methemoglobin, methylene blue and $O^{=}$, somewhat as follows:

Inductor + 4 MB → Oxidized inductor + 4 MBH_2 (leuco dye)
4 MBH_2 + $Hb(FeO_2)_4$ → $(MBH_2)_4$ $Hb(FeO_2)_4$ (complex peroxide)
$(MBH_2)_4$ $Hb(FeO_2)_4$ + 2 lactate → 4 MB + $Hb(Fe^{+++})_4$ + 2 pyruvate + 6 H_2O + 2 $O^{=}$.

Features included in this scheme: (1) An inductor (possibly unsaturated fatty acid or other easily oxidizable substance), the oxidation of which by methylene blue forms some CO_2 and the leuco-dye. (2) The reversible catalytic action of the dye. (3) The non-reversible catalytic action of the hemoglobin. (4) The stoichiometric relation between lactic acid oxidized and hemoglobin destroyed. (5) The sum of the O_2 required by these two processes is one half of the total O_2 consumption.

4888

The Mechanism of the Lethal Effects of Ultrasonic Radiation.

FRANCIS O. SCHMITT AND BERTHA UHLEMEYER.

From the Department of Zoology, Washington University, St. Louis, Mo.

That the lethal effects of ultrasonic waves on protozoa and other single cells could be traced to the cavitation of dissolved gas, has recently been discovered by Johnson.[1] Any gas suffices, apparently; there is no specificity of cavitated oxygen as was found for acceleration of chemical reactions by Schmitt, Johnson and Olson.[2] We have repeated the experiments of Johnson and are able to confirm his results fully. The present communication extends these observations and examines the mechanism of the lethal effect. A large number of microorganisms were tested, including various types of protozoa, rotifers, copepods, Daphnia, etc. In addition to these a number of eggs and embryos of frogs and snails were treated. About

[1] Johnson, C. H., *J. Physiol.*, 1929, lxvii, 356.

[2] Schmitt, F. O., Johnson, C. H., and Olson, A. R., *J. Am. Chem. Soc.*, 1929, li, 370.

10 cc. of the fluid containing the material to be tested was placed in a glass tube and radiated at atmospheric pressure, and at pressures of 60-80 lb. per square inch. After the minimum lethal dose for those radiated at atmospheric pressure was determined, it was found that an exposure many times this dosage had no effect upon cells radiated under 60-80 lb. pressure. Radiation of frog embryos yielded results which seem to indicate that radiation affects the head region rather preferentially, and that there is a definite selective interference in organogenesis. A study of these effects will be reported later.

To test the possibility that the function of the cavitated bubbles is to reflect the waves from their surfaces and thus tend to concentrate the radiation locally, two types of experiments were performed. In the first, cells were radiated in a suspension of infusorial earth, the suspended particles ranging from very small to relatively coarse. In the second, paramecia were allowed to ingest Chinese black which packed the food vacuoles with solid granules. Radiation of the cells in either case, with pressure and without, gave no results which would lend support to the theory that such an artificial increase of surface, inside or outside the cell, aided in producing the lethal effects concerned. It seems, therefore, that the lethal effect is produced at the surface of the cavitated gas bubbles. Johnson is of the opinion that the effect on cells is external rather than internal, and to his reasons for so believing, we may add the fact that, owing to the greater viscosity of the protoplasm, there will be considerably less tendency for cavitation inside the cell than outside. Thus, for example, when cells are radiated in a viscous medium, the minimal lethal dose is greatly increased. This retarding effect of viscosity is observable also in the promotion of chemical reactions by ultrasonic radiation.

To test the view that the lethal effect is due to a chemical or physical change at the plasma membrane, Spirogyra filaments were treated with a radiation strength insufficient to rupture the cellulose wall, or to cause visible disorganization. The strands were stained with neutral red, and then placed in a mixture of NaOH, NaCl, and $CaCl_2$. Radiated material gave evidence of a much more rapid penetration of the alkali than unradiated controls. Although adequate temperature control is difficult there seems little doubt but that the radiation alters the membrane so as to make it more permeable.

In further testing this view, another interesting and suggestive experiment was performed. Artificial cells were made by mixing a chloroform solution of lecithin with a protein solution according to

the directions of Harvey.[3] After the chloroform has diffused out of the "cells", an aqueous mixture of lecithin surrounded by a denaturized protein membrane presumably remains. Radiation of these "cells" gave very curious results. Sufficiently long exposures, of course, destroy the "cells". A dose of 10 seconds, however, sufficing to kill all paramecia present, results only in the accumulation of a bubble due to the cavitation of air inside the "cell"; successively longer radiations cause the appearance of protrusions, which in reality, are myelin forms resulting from the escape of lecithin into the water through ruptures in the cell wall. Microscopic examination occasionally reveals minute breaks in the membrane. That radiation may almost instantly break certain unstable emulsions, such as oils in water, and cause a separation of phases, has been demonstrated by Schmitt.[4] This seems highly significant in view of the Clowes theory of the emulsion structure of the membrane. It has also been discovered[4] that types of chemical reactions other than oxidations may be promoted by radiation in the presence of water and cavitated gas. Thus the hydrolysis of carbon tetrachloride and other halogens requires only cavitation; for this, pure nitrogen suffices.

The membranes of artificial cells may not be ruptured even though cavitation has taken place on the inside and the outside by radiation strong enough to kill all protozoa present; one seldom, if ever observes a cavitated bubble inside of radiated protozoa; very energetic chemical reactions may be promoted by radiation, when cavitation is permitted, even in the absence of oxygen; unstable emulsions may be instantly cracked by the radiation. These facts render plausible our tentative position that the lethal effect is due to a rupture of the plasma membrane by a chemical or a physical chemical effect produced by cavitation in the water immediately surrounding the cell.

[3] Harvey, E. N., "Laboratory Directions in General Physiology," 1913, 31.
[4] Schmitt, F. O., in press.

4889

Effect of Potassium Iodide on Basal Metabolism in Guinea Pigs.

WALTER J. SIEBERT AND ROBERT S. SMITH. (Introduced by Leo Loeb.)

From the Department of Pathology, Washington University School of Medicine, St. Louis, Mo.

Marine, Deutsch, and Cipra,[1] using an open circuit respiratory calorimeter of the Haldane type,[2] found that KI feeding to 18 normal rabbits caused a drop in the metabolic rate in 5, a slight increase in rate in 2, and no appreciable change in 11. Employing the same apparatus, Webster and Chesney[3] found, following the first dose of Lugol's solution, a decrease in basal metabolism in rabbits, which attained a maximum on the third day, with a subsequent tendency to a return to normal. Cordonnier[4] in our laboratory, using an apparatus devised by Foster and Sundstroem,[5] found that oral administration of KI over a 30 day period produced no appreciable effect on the average basal metabolic rate in 10 normal guinea pigs, though there were individual variations both above and below the normal averages.

Loeb,[6] Loeb and Gray,[7] and Rabinovitch,[8] have studied the effects of KI feeding upon compensatory hypertrophy and upon the structure of the thyroid gland in guinea pigs. They found that there is a marked increase in the number of mitotic figures and a slight softening of the colloid, with a slight increase in the height of the epithelium at a certain time during the period of feeding. Inasmuch as the apparatus used by Cordonnier gave somewhat variable results we decided to repeat the study of the effect of KI on basal metabolism in guinea pigs using the Haldane apparatus,* which gives much less variable results in control animals.

In 8 control guinea pigs weighing between 340 and 500 gm., we found that the basal rate of metabolism of the individual animal,

[1] Marine, D., Deutsch, M., and Cipra, A., PROC. SOC. EXP. BIOL. AND MED., 1927, xxiv, 657.

[2] Haldane, J. A., *J. Physiol.*, 1892, xiii, 419.

[3] Webster, B., Chesney, A. M., *Johns Hopkins Hosp. Bull.*, xliii, 291.

[4] Cordonnier, J., PROC. SOC. EXP. BIOL. AND MED., 1929, xxvi, 636.

[5] Foster, G. L., and Sundstroem, E. S., *J. Biol. Chem.*, 1927, lxix, 565.

[6] Loeb, Leo, *J. Med. Research*, 1920, xli, 481; *Am. J. Path.*, 1926, ii, 19.

[7] Loeb, Leo, and Gray, S. H., *Am. J. Path.*, 1928, iv, 257.

[8] Rabinovitch, Jacob, *Am. J. Path.*, 1928, iv, 601.

* We are indebted to Dr. Ethel Ronzoni for advice and assistance in the use of the apparatus.

recorded in calories per kilo per hour, varied within ±7.5% of the average rate for that animal. The average basal metabolic rates of these normal animals varied, however, between 3.22 and 4.09 calories per kilo per hour.

In 8 guinea pigs weighing between 340 and 500 gm. we found that daily oral administration of KI in 0.1 gm., 0.05 gm., and 0.01 gm. doses, over a 25-day period caused a definite, although not very great, increase in the basal rate of metabolism in 2 animals; a variation within the upper range of the normal metabolic values in one animal; a variation falling in the lower range of the normal metabolic curve in one animal; and, in 4 animals, variations in the basal metabolic rate corresponding to the average variations found in the controls. Of the 2 animals which showed an increased caloric output, one showed an increase of approximately 20% above the normal range of values in the first 10 days; the other showed a rise of approximately 10% above the range of variation of the controls, which occurred between the tenth and twentieth days. The average basal metabolic rate of all the KI fed animals was slightly above the average of the 8 control animals. All animals gained in weight throughout the experiment. No correlation was to be noted between the doses of KI given and the changes in the basal metabolism.

Our results, therefore, agree essentially with those of Cordonnier. From our determinations, it would appear that in guinea pigs, as in rabbits, the effect of oral administration of KI on the basal metabolic rate may be variable, and is not very great. In our experiments, however, KI feeding in guinea pigs tended to cause a slight increase in the basal metabolic level, rather than a decrease.

4890

Titrimetric Measurement of Fermentation.

MICHAEL SOMOGYI.

From the Laboratory of the Jewish Hospital of St. Louis, St. Louis, Mo.

The fact that yeast in high concentration ferments glucose very rapidly has been put to considerable use in the analysis of blood and other biochemical material. The fermentation can be so regulated that small quantities of glucose are completely broken down in a few minutes at room temperatures. The evolution of CO_2 takes

place so rapidly that the red color of phenol red (indicator) in a weakly alkaline glucose-yeast mixture turns to yellow in a few seconds.

In an attempt to apply this phenomenon as a means of quantitative observations in the study of fermentation processes, several serviceable procedures were tried out. The one to be described promises usefulness as the basis of a rather simple analytical technique.

The reagents employed are: (a) 0.01 molecular sodium carbonate solution, (b) 0.06% aqueous solution of phenol red, and (c) a 20% yeast suspension prepared by rubbing up 20 g. of commercial baker's yeast in water and making up the volume to 100 cc.

The procedure is as follows: Measure into a test tube 5 cc. of the sugar solution to be studied. Add one drop of phenol red and adjust the reaction to slight alkalinity (pink color, pH 7.2 to 7.4). Introduce into each of 2 other test tubes 5 cc. of the yeast suspension and 2 drops of phenol red, and add dropwise of the standard sodium carbonate solution until a distinct pink color persists for at least 30 seconds. To one of the yeast tubes 5 cc. of neutral (or neutralized) water are added; this serves as blank. The content of the other yeast tube is transferred into the tube containing the sugar, and the 2 fluids are immediately mixed by inversion of the stoppered tube. The pink color begins to fade in a few seconds, and at this point the titration must begin. A few drops at a time of the standard sodium carbonate solution are run in from a burette and mixed with the fluid by inversion of the tube. The rate of the titration must be so conducted as to keep the color of the indicator near the initial pink shade, and allow it at no time during the procedure to turn yellow. The end of the titration is indicated by the persistence of the initial pink shade for no less than 30 seconds after the last addition of sodium carbonate.

Meanwhile the color in the blank yeast has faded somewhat owing to the CO_2 produced by the respiration of the yeast. This is now titrated until the original pink color is restored. Deduction of this blank titration from the first titration figure, gives the titration value corresponding to the carbon dioxide derived from the sugar alone.

The procedure as outlined is but an example of the application of a rather flexible method, alterable to suit various experimental conditions and aims. With its aid it is possible to study the effect of a number of factors (concentration of yeast and of sugar, the sugar/yeast ratio, temperature, etc.) with greater facility and rapidity than is afforded by either the gasometric measurement of carbon dioxide or the usual methods of sugar determination.

TABLE I.
Amount of 0.01 Molecular Na_2CO_3 Solution Consumed in the Titration of CO_2 Formed When 5 cc. of Glucose Solution Are Fermented with 5 cc. of 20% Yeast Suspension.

1	2	3	4	5
		0.01 molar Na_2CO_3 solution consumed for titration of		
Amount of glucose	Time used for titration	CO_2 formed in yeast-glucose mixture	CO_2 formed in yeast suspension without glucose	CO_2 formed from glucose (Column 3 minus col. 4)
mg.	*minutes*	*cc.*	*cc.*	*cc.*
5	5-6	0.46	0.05	0.41
10	6-7	0.85	0.05	0.80
15	8-10	1.26	0.06	1.20
20	9-11	1.67	0.07	1.60
25	11-12	2.11	0.07	2.04

Table I is presented as an example of experiments carried out with our procedure. The results recorded, each representing the average of 3 closely agreeing titrations, show a linear proportionality between glucose concentration and titration figures, with yeast concentration, total volume and temperature kept unchanged. The CO_2 produced may be calculated from the equation

$$pH = 6.1 + \log \frac{[BHCO_3]}{[\text{free } CO_2]}$$

The value of $BHCO_3$ is known from the total amount of sodium carbonate introduced, the pH is indicated by the color of the mother liquor.

In possession of the titration equivalents of known amounts of fermentable sugars, it is possible to utilize this technique as an analytical procedure for the determination of sugars. In Table II are given some determinations of fermentable sugar in diabetic urine. Although we do not propose the use of this technique as an accu-

TABLE II.
Determination of Fermentable Sugar in Urine by a Fermentation-Titration Method.

No.	Shaffer-Hartmann method (modified)			Fermentation-titration method
	Total reduction	Non-fermentable reducing subst.	Fermentable sugar	
	%	%	%	%
1	1.42	0.16	1.26	1.26
2	2.64	0.17	2.47	2.30
3	0.79	0.21	0.58	0.58
4	4.66	0.11	4.55	4.58
5	0.47	0.08	0.39	0.38
6	1.31	0.19	1.12	1.20

rate quantitative method, it may become useful in the clinical laboratory, especially in cases where the distinction between glucose and non-fermentable sugars becomes necessary (lactosuria, pentosuria). The figures in Table II demonstrate that with a little practice fairly accurate results can be obtained. Since these results represent only fermentable sugar, for comparison the same was determined by the Shaffer-Hartmann method. Copper reduction methods in general furnish sugar values that are too high, since urine contains varying amounts of reducing substances other than sugar, thus the latter must be determined separately in the fermented urine and deducted from the total reduction in order to obtain the amount of fermentable sugar (glucose). It may be noted in the figures in Table II that by ignoring this fact and accepting total reduction values as sugar, the error inherent in copper reduction methods may exceed the experimental error attendant upon our simple semi-quantitative procedure.

The range of glucose concentrations recorded in Table I does not by any means represent the limits of applicability of this technique. It is effective with considerably higher as well as lower concentrations; in fact it is well possible to follow by titration with 0.001 molecular sodium carbonate the fermentation of as little as 0.5 mg. of glucose.

Southern Section.

Tulane University of Louisiana, March 2, 1930.

4891

Electrocardiographic Studies of Dogs Infested with Dirofilaria immitis.

S. N. BLACKBERG AND RICHARD ASHMAN.

From the Departments of Pharmacology and Physiology, Tulane University.

Electrocardiograms were taken of 3 dogs uninfested and of 2 infested by *Dirofilaria immitis*. No significant differences were found. One normal and 2 infested dogs were then exercised moderately, electrocardiograms being recorded shortly before and immediately after running for 10 min. on a treadmill. In the normal dog and in one infested, exercise produced no significant changes. In the other dog, however, the QRS interval was slightly prolonged (0.04 to 0.05 sec.); the R wave amplitude was decreased in all leads (from 16.0 mm. to 10.5 mm. in lead II); and the T wave in lead I was inverted (from an amplitude of 1.0 mm. upward to 1.0 mm. downward), while in lead II, T changed from a height of 2 mm. to zero or isoelectric.

The change in the T wave is like that observed in man during anginal attacks.[1]

[1] Feil, H., and Siegel, M. L., *Am. J. Med. Sci.*, 1928, clxxv, 255.

4892

Unidirectional Block in Cardiac Muscle.

RICHARD ASHMAN AND ROBERTA HAFKESBRING.

From the Department of Physiology, Tulane University of La.

In a previous paper[1] experiments were reported in which a unidirectional block was produced by asymmetrical compression applied at the middle of transverse strips from the turtle ventricle. It has since been found possible to induce a similar one-way block by means of 2 oblique cuts, starting from opposite points near the middle of the lateral edges of the strip and converging toward the center, but leaving a bridge connecting the two halves of the strip. Thus the central end of the one half of the strip is wedge-shaped. Responses to induction shocks applied to either end of the strip were recorded by the string galvanometer.

In 30 of 41 experiments, partial or complete block occurred at the bridge when the impulse was required to travel through the wedge before reaching the bridge. When the impulse came from the opposite end of the strip, there was either no blocking or the degree of block was definitely less. In 10 of the 41 experiments, the reverse result was obtained. In one experiment, conduction was equal in the two directions.

Although these results are much less conclusive than those previously reported, it seems clear that here also a similar juxtaposition of the more and of the less depressed muscle determines the direction in which blocking shall occur, namely, the impulse may be blocked if it invades the less before reaching the more depressed tissue; it is transmitted if the more depressed muscle is first to be invaded.

In those cases in which, with slower rates of stimulation, there was conduction in both directions, the impulse was usually transmitted more rapidly in the expected direction.

[1] Ashman, R., and Hafkesbring, R., *Am. J. Physiol.*, 1929, xci, 65.

4893

Formation of Agglutinins in Response to Routine Inoculation of Typhoid Vaccine.*

ROY F. FEEMSTER. (Introduced by C. W. Duval.)

From the Department of Pathology and Bacteriology, Tulane University of Louisiana.

The universality of inoculation of typhoid vaccine has rendered the Widal reaction perhaps even more unreliable for diagnosis than it has been in the past. For this reason a further study of the agglutinin response to typhoid vaccine would be of value in helping to interpret this diagnostic test.

Broesamlen[1] states that 74% of 482 healthy persons whom he followed showed positive agglutination after typhoid vaccine, and that 41% were still positive at the end of 2 years. Fennel[2] studied 30 individuals and came to the conclusion that previous vaccination repressed agglutination formation and that there was no relation between the local and systemic reaction and the agglutinin formation. It is interesting to note that the present study fails to confirm the conclusions of either of these workers.

Opportunity was afforded by the routine inoculation of the class in bacteriology against typhoid fever to study the formation of agglutinins in a group of 95 healthy young adults.

Samples of blood were taken from each student 10 days before inoculation and tested by macroscopic methods for the presence of agglutinins. A group of 25 were again tested just after the second inoculation. The final tests were performed on blood taken 10 days to 2 weeks after the third dose of vaccine. A history was obtained from each student as to whether he had had typhoid fever, previous vaccinations, and as to the type and severity of reaction to the inoculations. The data collected in this way were carefully analyzed statistically and the following conclusions seem to be warranted:

1. Agglutinins are developed more universally by those who have had typhoid (100%), or previous vaccination (83%) than by those who have had neither (45%).

2. Agglutinins are developed in higher titer by those who have had typhoid (1/242), or previous vaccination (1/163) than by those who have had neither (1/74).

* Credit should be given to two students of the class, H. A. King, Jr., and D. A. Savant, who performed a large part of the routine work.

[1] Broesamlen, *Deutsches Arch. f. Klin. Med.*, 1918, cxxix, 208.

[2] Fennel, E. A., *J. Am. Med. Assn.*, 1918, lxx, 1915.

3. Agglutinins from previous vaccinations persisted in titer high enough for diagnosis (1/40) in only 21% at the end of one year, and in only 12% at the end of 2 years.

4. There is some correlation, but not marked, between the severity of reaction to inoculation and the titer developed. (Correlation coefficient is $+0.393 \pm 0.058$.)

5. There is no significant correlation between the number of previous vaccinations and the titer developed. (Correlation coefficient is $+0.154 \pm 0.092$.)

6. There is no significant correlation between the time elapsed since the last vaccination and the titer developed. (Correlation coefficient is -0.105 ± 0.093.)

7. There was a steady rise in agglutinin titer in the group of 25 on whom 3 sets of tests were performed. (Typhoid group 1/3, 1/73, 1/276; previously vaccinated group 1/28, 1/59, 1/270; unvaccinated group 1/0, 1/50, 1/62.)

Further study of this group is contemplated. Parallel agglutination and complement fixation tests are to be run at the end of 3 months from inoculation.

4894

I. Relation of Bile Salts, Cholesterin, Sodium Citrate and Sodium Bicarbonate to Toxicity of Pneumococcus.

JOHN W. WILLIAMS. (Introduced by C. W. Duval.)

From the Department of Pathology, College of Medicine, Tulane University.

Experiments were performed to determine certain facts relative to the action of sodium citrate, sodium bicarbonate, and some of the constituents of the bile, namely cholesterin and sodium taurocholate, on the toxicity of the pneumococcus. These substances were used separately and in combinations and were injected intraperitoneally into the white mouse. In all, approximately 75 such mice were employed. It is thought by some that bile salts increase the toxicity of the pneumococcus and that cholesterin acts as a buffer reducing this toxicity. The results obtained by various workers[1,2,3,4]

[1] Rosenow, E. C., *J. Inf. Dis.*, 1911, ix, 190.

[2] Cole, Rufus, *J. Exp. Med.*, 1912, xvi, 664.

[3] Horrall, O. H., and Carlson, A. J., *Am. J. Physiol.*, 1928, lxxxv, 59.

[4] Ravdin, I. S., Morrison, M. E., and Smyth, C. M., *Ann. Surg.*, 1929, lxxxix, 871.

have, however, not agreed in all features and for that reason it seems justifiable to place on record all experiments pertinent to this subject.

Previous workers[4] have reported that when sodium taurocholate was dissolved in distilled water and injected into the peritoneal cavity of the mouse, the largest dose tolerated was 0.009 gm. In those experiments in which normal saline was used in place of distilled water the sodium taurocholate product* showed approximately this toxicity. We, therefore, employed one-half the lethal dose or 0.005 gm. of the product.

In comparison with sodium taurocholate dissolved in normal saline, a like solution with 1% sodium citrate added, was made up and it was found that the toxicity was practically doubled while the sodium citrate injected alone had no toxic effect. Twenty-four hour growths of the pneumococcus, Type I, on blood agar were washed down with sterile saline. Suspensions and dilutions thereof were injected into a number of mice in varying dosage in order to standardize the virulence.

To determine the relationship of the number of microorganisms to their virulence, a dilution of 1:8 was made. While 0.05 cc. of the undiluted suspension killed in 18 to 24 hours, equal amounts of the diluted suspension produced death in one-half of the animals in 48 hours. If 0.005 gm. of sodium taurocholate was added to the undiluted suspension the animals died in 8 to 12 hours, whereas if it was added to the diluted suspension the animals died in 20 to 40 hours.

A series of injections of pneumococcus I and sodium taurocholate was made. One-half cc. of the pneumococcus suspension and 0.005 gm. of sodium taurocholate caused death of the animals in 8 to 12 hours. On the other hand, if the sodium taurocholate was injected first and the pneumococcus 10 minutes later, the animals died in 10 to 14 hours; if the pneumococcus was injected 4 hours later the animals likewise died in 10 to 14 hours. Control animals injected with pneumococcus alone died in 18 to 24 hours.

In the next series it was attempted to determine the effect of cholesterin on the toxicity of sodium taurocholate and the pneumococcus. 0.005 gm. of sodium taurocholate, saturated with cholesterin, were added to the pneumococcus suspension and the injected animals died in 8 to 14 hours. However, in 2 animals in which 5% sodium bicarbonate was added to the sodium taurocholate saturated with cholesterin one animal lived for 48 hours and the other survived. Control animals injected with a suspension of cholesterin in saline

* Merck & Co., about 47% pure sodium taurocholate.

alone and with sodium bicarbonate were unaffected while animals injected with sodium taurocholate saturated with cholesterin showed practically the same degree of toxic effects as those injected with sodium taurocholate alone.

As a comparative observation 2% sodium taurocholate dissolved in normal saline was used in conjunction with the pneumococcus Types I and III on the one hand and 1% sodium citrate and 2% sodium taurocholate dissolved in normal saline in conjunction with the pneumococcus Types I and III on the other. In the latter instance death occurred in 18 hours with Type I and in 18 to 50 hours with Type III, while in the former instance death occurred in 26 hours with Type I and survival resulted with Type III.

To determine whether a toxic filtrable substance might be formed upon the addition of sodium taurocholate to pneumococcus suspension, varying dilutions of each were made and incubated from 10 minutes to an hour in order to allow any change which might be effected. In no instance did we obtain after passage of this preparation through a Berkefeld filter a filtrate which manifested any toxic effect when injected intraperitoneally into the mouse.

4895

II. Comparison of Inulin Bile Titration and Virulence of Pneumococcus.

JOHN W. WILLIAMS. (Introduced by C. W. Duval.)

From the Department of Pathology, College of Medicine, Tulane University.

As it might be possible to determine the variation of the virulence of the pneumococcus by means of the extent and rapidity of fermentation of inulin and the effect of different dilutions of bile salts on this reaction, a series of observations were carried out.

Sodium taurocholate was the bile salt chosen and in certain instances other substances such as cholesterin, sodium citrate and sodium bicarbonate were also incorporated in the solution. Normal saline was used as a vehicle throughout. A 24 hour culture of the pneumococcus was washed down with normal saline and transplants made from this suspension in inulin were incubated for 24 hours. Pneumococcus I was used routinely and type III merely employed at times in comparison. In all instances cultures were obtained from our laboratory stock supply.

In the first trials, the inulin media used contained no peptone but it was soon found that in many instances where the virulence of the microorganism was impaired, redness but no coagulation would occur. When peptone was added it apparently afforded a nidus for the enzymatic action of the pneumococcus and caused coagulation in many of the instances wherein it had not occurred previously. One-fourth per cent peptone was used thereafter as it had been found that equally good coagulation was produced on the addition of a trace to 2% of this substance.

The time of fermentation at 37°C. was next determined and it was noted that redness began to occur within 6 hours and that coagulation was complete in 17 hours. When, however, the peptone was omitted both the rapidity and the intensity of the reactions were decreased.

Observations were made with relation to the effect of the number of pneumococci transplanted into the inulin. It was found that coagulation ceased in dilutions of 1:48 of the prepared suspension but that redness continued in dilutions as high as 1:49,000. The point of this transition depended upon the virulence of the pneumococcus and its type, since III did not lose its ability to ferment inulin to anywhere near the degree of pneumococcus I in proportion to its loss of virulence.

Upon keeping the pneumococcus in stock for 4 months the loss of virulence of type I was accompanied by a loss of ability to ferment inulin. It was also found that pneumococcus III which lost much of its virulence was less markedly affected in its ability to ferment inulin and the relative comparison of the reactions was lost.

In the subsequent tests the addition of sodium taurocholate dilutions to transplants of pneumococcus I in inulin, employing a constant suspension, demonstrated a curve with successive dilutions of the bile salt of redness, redness and haziness, redness and milkiness, and coagulation wherever the virulence of the microorganism was not too much impaired. This curve seemed to shift to a higher dilution in accordance with the decrease in toxicity of the pneumococcus for the white mouse. The lowest dilution showing a reaction was 1:400 of the sodium taurocholate.

In addition to the sodium taurocholate solution, 1% sodium citrate in the 2% sodium taurocholate solution was used and it was found that the pneumococcus showed a result in the next higher dilution which is in accordance with the increased virulence shown by this mixture for the white mouse and the increased toxicity of this solution.

Two other substances used in solution with the sodium taurocholate were cholesterin and sodium bicarbonate. Cholesterin showed no change in the dilution at which reaction of the pneumococcus on inulin took place when sodium taurocholate alone was used. Sodium bicarbonate even in the higher dilutions of 1:65,600 prevented a change in the inulin which was due, in all probability, to its alkalinity. This change was out of proportion to the slight protective effect it exerted on the white mouse.

Pneumococcus II of our stock was run as a comparison and was found to possess no virulence and to show no reaction on inulin.

Pneumococcus III, as stated previously, did not lose its ability to ferment inulin in the presence of bile dilutions in proportion to its loss of virulence. An interesting point, however, presented itself with regard to its coagulation in that it started in the bottom of the tube while that of pneumococcus I started in the top of the tube. In this type of pneumococcus sodium citrate in sodium taurocholate also shifted the reaction to the next higher dilution.

In summarizing the results, it appears that the ability of pneumococcus I to ferment inulin, especially when the bile salt dilution is added, varies with its virulence for the white mouse and its numbers, while that of pneumococcus III maintains its ability to ferment inulin on loss of virulence to a much greater degree than type I. There is also demonstrated an inhibiting effect on pneumococcus inulin fermentation, if the sodium citrate is added to the sodium taurocholate solution which is in accordance with the increased toxicity of this solution for the white mouse.

4896

A Comparative X-Ray Study of Passage of Foodstuffs Through Gastro-intestinal Tract of Rats.

L. J. MENVILLE, J. N. ANÉ AND S. N. BLACKBERG.
(Introduced by J. H. Musser.)

From the Department of Medicine and Department of Pharmacology, Tulane University.

For many years previous to the discovery of the Roentgen ray, studies of the emptying time of various foodstuffs by the stomach and intestines were made by many investigators. These early investigations were usually conducted on animals with artificial fistulas. It was impossible at that time to visualize the viscera of these ani-

mals except by operation, and this was undesirable as the process of digestion was thereby interfered with.

Cannon[1] was the first to employ the Roentgen ray in such a study. His observations were made on healthy animals, principally cats. The Roentgen ray afforded him the opportunity to visualize and study the digestive tract of these animals uninfluenced by any artificial conditions which could disturb their digestion. His studies were undertaken a few years after the discovery of the Roentgen ray when little was known of the normal motility of the gastro-intestinal tract.

It occurred to us that such a study at this time, using improved Roentgen ray technic and the modern powerful apparatus together with the present day knowledge in this field relative to the gastro-intestinal tract would yield more satisfactory results. For this reason, we undertook the study of the passage of carbohydrate, protein and fatty foodstuffs through the gastro-intestinal tract of rats in order that the findings of the earlier investigators may be compared with the results obtained through the employment of present modern facilities.

In our experiments, 39 healthy rats were used. These rats before being examined were fasted for 48 hours and deprived of water for 24 hours. The animals were placed in the same environment and handled in the same manner as reported by us[2] in our observations upon rachitic rats.

Two separate sets of carbohydrate and protein fed rats and one set of fat rats were employed. There were 14 rats in the first carbohydrate group and one in the second group. This rat was used as a check on the rats of the first group as it was not found necessary to use more inasmuch as the emptying times of the gastro-intestinal tract of the first group were in accordance with the findings of other observers. The rats of both groups were fed 7½ gm. of starch and 2½ gm. of barium sulphate for 20 minutes. They were immediately fluoroscoped in loose cotton sacks to ascertain if the stomach was full and at the same time to observe if any of the food was passing through the pylorus. Careful fluoroscopic observations were made every 15 minutes thereafter until it was definitely demonstrated that the food column was entering the cecum.

In the instance of the rats fed with protein our findings were in variance with other investigators and for this reason we ran 2

[1] Cannon, W. B., *Am. J. Physiol.*, 1904, xl, 416.

[2] Menville, L. J., Blackberg, S. N., and Ané, J. N., PROC. SOC. EXP. BIOL. AND MED., 1929, xxvi, 758.

groups, the second serving as a check on the first group. In the first protein group we used 10 rats to which we fed 7½ gm. of casein and 2½ gm. of barium sulphate. In the second group we used 8 rats, giving them the same food in the same quantity as in those of the first group. Both groups were permitted to feed for 20 minutes and were fluoroscoped in the same manner as those of the carbohydrate groups.

Six rats composed the fat group and they were fed 7½ gm. of pure cream mixed with 2½ gm. of barium sulphate and permitted to feed for 20 minutes and examined radiologically as in the carbohydrate and protein groups.

The various foodstuffs ingested by all of the rats were of approximately the same general consistency.

TABLE I.

General Averages of Series No. 1.

Food	Wt. Gm.	Ate Gm.	Cecum Appearance Time		Stomach Emptying Time		Small Intestine Emptying Time		Colon Emptying Time	Rats No.
			hr.	min.	hr.	min.	hr.	min.	hr.	
Carbohydrates	204.4	5.2	5	4	6	56	10	11	53	14
Proteins	275.2	7.56	4	12	11	58	14	06	63.4	10
Fats	235.8	5.3	8	35	16	44	29	10	61.3	6

TABLE II.

General Averages of Series No. 2.

Food	Wt. Gm.	Ate Gm.	Cecum Appearance Time		Stomach Emptying Time		Small Intestine Emptying Time		Colon Emptying Time	Rats No.
			hr.	min.	hr.	min.	hr.	min.	hr.	
Carbohydrate	200	6	5	23	9	8	13	30	....	1
Protein	207.5	6	4	42	14	32	16	10	....	8

TABLE III.

Compiled Averages of Series No. 1 and No. 2.

Food	Wt. Gm.	Ate Gm.	Cecum Appearance Time		Stomach Emptying Time		Small Intestine Emptying Time		Colon Emptying Time	Rats No.
			hr.	min.	hr.	min.	hr.	min.	hr.	
Carbohydrate	204.1	5.3	5	05	7	05	10	24	53	15
Protein	245.1	6.8	4	26	13	09	15	01	63.4	18
Fats	235.8	5.3	8	35	16	44	29	10	61.3	6

The first fluoroscopic observations were made on the rats of all groups immediately after the completion of the feeding time and in every instance the stomach was found filled and some of the food had already passed through the pylorus. This finding is in accord

with that of McClure, Reynolds and Schwartz.[3] Careful fluoroscopic observations were then made every 15 minutes until the food column was visualized in the cecum and the stomach and small intestine was found empty. Skiagraphs were made of representative rats of the various groups, so that they may serve as records of the results. The fluoroscopic examinations of the emptying time of the colon were made at longer intervals which varied from one hour to several hours. The results obtained in all of these observations are recorded in our tables.

As shown in Table No. I, the average emptying time of the stomach of the carbohydrate group was the most rapid, the protein next, while the fat was the slowest of all. The cecum appearance time of the food column of the protein group was the fastest, next the carbohydrate and last the fat group. The small intestine emptying time of the carbohydrate group was the fastest, next the protein and last the fats. The colon emptying time of the carbohydrate group was the fastest of all and with a slight difference between the protein and fat groups. These observations on the colon are not as accurate as those of the stomach and small intestine because this organ empties itself very slowly of the various foodstuffs used and for this reason the fluoroscopic examinations were of longer intervals.

Table II represents a check on the group as shown in Table I and it is seen that the findings in Table II check closely with those in Table I.

Conclusions. These experiments demonstrate that carbohydrates in the form of starch, protein in the form of casein, and fat in the form of pure cream all leave the stomach of rats at approximately the same time. The rate of emptying time of stomach, small intestine and colon corresponds closely to those found by other observers. Although the emptying times observed by us were approximately the same as those of previous workers, marked differences were noted for the rates of passage of the various foodstuffs in our experiments as compared with others.

The passage of protein through the small intestine of rats is much faster in every instance than fat and only slightly faster than carbohydrate. This is at variance with the findings of the earlier investigators, who stated that proteins pass through the small intestine slowly, fats a little faster and carbohydrates fastest of all.

We believe that the rate of the food column of the different foodstuffs through the small intestine is probably due to the muscular

[3] McClure, C. W., Reynolds, L., Schwartz, C. O., *Arch. Int. Med.*, 1920, xxvi, 410.

response of this organ. The rate of the emptying time of the stomach seems a negligible factor as it was demonstrated that some of the meal of the different foodstuffs passed through the pylorus at about the same time.

4897

Is the Cystic Bile Resorbed in Toto?

ELEANOR A. HUNT AND E. A. BOYDEN.

From the Department of Anatomy, University of Alabama.

Notwithstanding the great body of evidence accumulated during the last 7 years tending to show that the gall bladder discharges the bulk of its contents after meals, it has lately been reaffirmed that the primary function of this organ is to resorb the cystic bile *in toto* for the purpose of returning it to the general circulation, and that "under normal conditions, whatever passes into the gall bladder through the cystic duct, never passes out again through the cystic duct."[1]

Although this hypothesis has had the effect of stimulating research in a difficult field, it owes its existence largely to teleological reasoning. Thus, to one investigator it offers the best theory for explaining the formation of gall stones and the occurrence of hydrops of the gall bladder[1]; to another, it is justified by the assertion that no one has explained why a biliary reservoir is necessary for digestion[2]; to a third the interpretations of the results of cholecystography are easier and the manifestations less contradictory if it is assumed that the bile enters the gall bladder not to be stored there and in time expelled, but to be resorbed *in toto* by the gall bladder mucosa.[3]

Yet it is to be questioned whether, in the 7 years that have elapsed since this theory was first promulgated, any proof that the cystic bile is resorbed *in toto* has ever been advanced. Believing that this theory is susceptible to proof or disproof we have undertaken to block the cystic duct in cats in such a way as to avoid trauma to the gall bladder or interference with its vascular drainage—a method successfully employed by both Sweet and Halpert in their experiments. With the outlet blocked it is reasonable to expect that the gall blad-

[1] Halpert, Bela, *Arch. Surg.*, 1929, xix, 1037.

[2] Sweet, Joshua, *Annals Surg.*, 1929, xc, 939.

[3] Blond, Kasper, *Arch. f. klin. Chir.*, 1928, cxlix, 662.

der would entirely empty itself by absorption within one to 3 days, if the above theory is correct. That such is not the case is indicated by the following experiments.

If a cat be fed a meal of meat on the evening before an operation, the gall bladder will be found distended with light green, watery bile the next morning. Since in most cats the cystic duct is convoluted, a bend can be selected for ligation which lies to one side of the main cystic artery, vein and lymph channels. At the convexity of such a bend the duct can be ligated without causing hemorrhage or interruption of vascular drainage. (Incidentally, there are usually accessory veins on the gall bladder that drain directly into the liver). Also, if cat-gut be employed, ligation can be accomplished under sterile conditions without causing noticeable inflammation of the duct or peritoneal adhesions. Forty-eight hours later (during which interval the animal has demonstrated perfect recovery from the operation) examination of the cat under anesthesia shows that the gall bladder has diminished from one-half to one-third of its original volume and that inspissation has changed its contents from a light green, watery fluid to a dark green, semi-solid mass. Furthermore, application of the Pettenkofer test indicates the presence of large quantities of bile salts in the unabsorbed residue.* Thus, while the water content of the bile has been largely removed, neither bile pigment nor bile salts have been efficiently resorbed in this long interval.

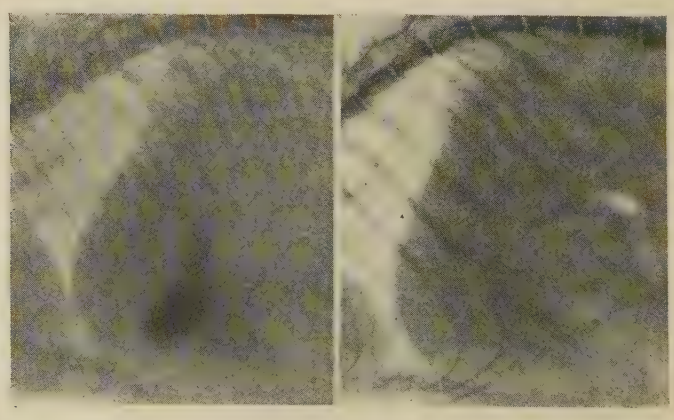


FIG. 1.

Left hand picture shows X-ray of gall bladder of Cat A-21 two hours after gall bladder was filled with a 10% solution of sodium iodide. Right hand picture, taken an hour and a half later, shows disappearance of shadow, following absorption of the iodide.

* For these chemical tests we are indebted to Professor E. B. Carmichael of the Department of Physiological Chemistry.

Further evidence that the absorptive capacity of the gall bladder has not been impaired by operative procedures may be demonstrated in the following way. If at the end of 48 hours the inspissated bile is aspirated from the fundus of the gall bladder, and replaced with 2 cc. of 10% solution of sodium iodide, X-rays of the living animal will reveal the disappearance of the iodine shadow within 3 hours after the operation (Fig. 1).

These experiments thus confirm the observation of Sweet[2] that sodium iodide is absorbed by the mucosa of the *vesica fellea,* but even more they indicate the difficulty of maintaining that the contents of the gall bladder are resorbed *in toto* or that the primary "function of the gall bladder is to receive and return to the body the bile which is formed during the intervals between active digestion."

4898

Reaction of the Gall Bladder to Stimulation of Visceral Nerves.

EDWARD A. BOYDEN.

From the Department of Anatomy, University of Alabama.

In a recent article[1] evidence was presented to show that both human and animal gall bladders are subject to the control of reflexes originating in the gastro-intestinal tract; that in unanesthetized cats faradic stimulation of the *pars pylorica* of the empty stomach induces contraction of that organ and reflex emptying of the gall bladder; and that faradic stimulation of any part of the alimentary tube from stomach to caecum will inhibit the contraction of a gall bladder that is emptying after a meal of egg-yolk.

Since then, employing similar methods (*i. e.,* sewing insulated electrodes to various internal surfaces) these observations have been extended to the colon and rectum of the cat (3 animals), and to the peritoneal surface of the abdominal musculature (1 animal), thereby showing that faradic stimulation of certain parts of the gut-tract or body-wall that are supplied by sacral and thoracic nerves, respectively, also induces inhibition of the emptying gall bladder.

Using a modification of this method, by means of which electrodes may be wrapped around nerve trunks and insulated from surrounding parts, it has been found possible to stimulate the nerves of the

[1] Boyden, E. A., and Birch, C. L., *Am. J. Physiol.,* xcii, 287; Birch, C. L., and Boyden, E. A., *Ibid.,* 301.

unanesthetized animal after its recovery from the operation and thereby to induce changes in the tonus of the gall bladder as revealed by X-rays of its roentgen-opaque contents. Preliminary attempts to ascertain the nerves involved in these reflex pathways have demonstrated that direct stimulation of the plexus that accompanies the left gastric artery (2 animals), or of the hepatic plexus (1 animal), induces relaxation of the contracted gall bladder.

This preliminary report is based on 14 experimental animals. In the 8 animals in which the conditions prerequisite to inhibition were established (*i. e.*, emptying of the gall bladder following ingestion of egg-yolk), faradic stimulation sufficient to cause slight pain, repeatedly and consistently stopped the flow of cystic bile.

4899

A Method for Obtaining a Pure Culture of Balantidium coli.

ERNEST CARROLL FAUST.

From the Parasitology Laboratory, Department of Tropical Medicine, Tulane University of Louisiana.

Balantidium coli, the ciliate of the large bowel of the pig, and not infrequently reported from man, the monkey and the guinea pig, has been grown *in vitro* by Walker,[1] Barrett and Yarbrough[2] and Rees.[3] The latter investigator was able to isolate single individuals and start a pure line of the organism obtained from the guinea pig. In all of these attempts, however, the medium was contaminated by various fecal bacteria and no efforts appear to have been made to develop pure cultures, although the need for such a procedure is evident.

Opportunity to attempt the sterilizing of balantidia against fecal bacteria was presented to the writer during the summer of 1929, when all of 12 individuals of *Macacus rhesus,* which had been under observation by Dr. C. C. Bass for a non-protozoan infection, were found to be passing cysts and trophozoites of *Balantidium coli* in their stools. Rectal specimens from those individuals which gave the richest yields of trophozoites were washed and concentrated in a

[1] Walker, E. L., *Phil. J. Sci.,* (B), 1913, viii, 1.

[2] Barrett, H. P., and Yarbrough, N., *Am. J. Trop. Med.,* 1921, i, 161.

[3] Rees, C. W., *Science,* 1927, lxvi, 89.

modified Ringer's fluid and the active organisms freed of fecal debris and gross bacterial contamination.

Preliminary tests were then made with several bactericidal agents in various dilutions to determine if any could be found which were lethal to the bacteria without injuring the active balantidia. The most promising of these were acriviolet and neutral acriflavine. The washed trophozoites were placed in the following dilutions of each of these drugs and the organisms examined after 5 minutes, 10 minutes, 30 minutes and 60 minutes respectively: 1/1,000; 1/2,000; 1/4,000; 1/8,000; 1/16,000; 1/32,000; 1/64,000; and 1/128,000. In both series the dilutions of 1/8,000 or less, even for as short a period as 10 minutes were toxic to the trophozoites. Those in acriviolet at a 1/16,000 dilution or greater survived for 10 minutes but were killed before 30 minutes. Those in acriflavine survived and were active in a 1/16,000 dilution or greater for 30 to 60 minutes. In acriviolet the dead organisms were violet stained; in acriflavine they were orange tinted; in both media the active organisms were tinted a delicate lemon hue. The living organisms were removed under sterile precautions to various culture media where they immediately began to feed. Fractions from the material submitted to the various dilutions of the two dyes for 30 minutes were placed on glucose-agar slants and incubated at 37°C. They were all negative for bacteria and yeasts, except the 1/64,000 dilutions, in which a few colonies developed and the 1/128,000 dilutions in which abundant growth took place.

Since the 30-minute contact with the dye was most convenient for manipulation and since acriflavine proved to be less toxic for the balantidia for this amount of exposure, the experiment was repeated several times, with dilutions of 1/1,000; 1/5,000; 1/10,000; 1/15,000, etc., up to 1/70,000. It was found that healthy trophozoites could stand exposure in all dilutions from 1/70,000 to 1/20,000 for 60 minutes; in dilutions to 1/15,000 for 30 minutes; in dilutions of 1/10,000 for 10 minutes, and in dilutions of 1/5,000 for 5 minutes or less. Glucose-agar inoculations from all of these were negative up to 10 days, when the culture tubes were discarded.

These experiments indicate that acriflavine is capable of sterilizing trophozoites of *Balantidium coli* against bacteria and yeasts in dilutions well within the viable limits of the ciliate. One probable reason for this success was the thorough washing of the balantidia in an isotonic medium preceding exposure to the dye, thus freeing them from gross fecal contamination. The faint lemon tinting of the balantidia and even the staining of the nuclei apparently produced no unfavorable effect on the organisms, since they proceeded

to feed, at times ravenously, as soon as they were removed to a sterile nutrient medium. This same method may possibly be useful for the sterilization of the trophozoite stage of other intestinal protozoa.

4900

A Standardized Method for Pollen Air Analysis.*

W. T. PENFOUND AND B. G. EFRON. (Introduced by Isadore Cohn.)

From the Botany Department of Tulane University and the Allergy Clinic of Medical Department of Touro Infirmary and Hay Fever Clinic of Senses Hospital.

Pollen air analysis has come to play an important rôle in the diagnosis and treatment of allergic diseases caused by weeds. The usual method of determining the amount of wind-borne pollen is as follows: A portion of a glass slide is marked off near the center and thinly coated with vaseline, glycerine, or corn oil. It is then placed in a horizontal position or at an angle in some exposed situation for 24 hours. The slide is taken to the laboratory in a closed container, and the pollens on the ruled portion are identified and counted with the aid of a mechanical stage and a micrometer ocular.

Comparisons of slides placed in various sections and situations in New Orleans throughout 1929 revealed a great difference in the numbers and percentage compositions of pollen of various species. Very high counts were often obtained in the heart of the business district, and at the same time low counts prevailed in the residential and suburban districts. Slides placed on different sides of the same building at the same time, showed marked discrepancies in both numbers and species of pollen. In addition, relatively low counts were obtained during periods of marked hay fever, and *vice versa.* These results were, no doubt, directly related to the degree of exposure.

This diversity in the percentage of pollen of a given species in a given situation raised the question as to whether one might not get a great difference on slides placed in one location simultaneously, and exposed in positions varying from the horizontal to the vertical. Accordingly, slides were exposed at various inclinations with the horizontal and counts were made. The vertical slides showed more pollen on windy days, provided they were exposed perpendicular to

* This work was aided by a grant from the Harry Dennery Research Fund.

New York Meeting.

New York Academy of Medicine, April 16, 1930.

4901

An Accurate and Practical Method for Blood Platelet Counting.

ALBERT E. CASEY AND OSCAR M. HELMER. (Introduced by L. Pearce.)

From the Laboratories of The Rockefeller Institute for Medical Research.

A new method of blood platelet counting involving no additional technical procedure has been devised which has been found to be as accurate and as rapid as the usual method of red cell enumeration. Its essential features are: (1) the red blood cells and blood platelets are counted on the same field in the same counting chamber preparation; (2) the low power lens is used for both red and platelet counts; (3) the platelets are counted in the 3 middle vertical columns of a Neubauer chamber, comprising 240 small squares and the resultant figure divided by 3; (4) Ringer's solution, to which a small amount of heparin (1 mg. per 5 cc.) has been added, is used as the diluting fluid.*

Parallel counts indicate that as accurate red cell counts may be obtained by this method as with Hayem's solution. In a series of consecutive pipettes upon the same animal, the mean red blood count was 5,042,500 and the mean platelet count 536,000. The coefficient of variation for the red counts was 6.8% and for the platelets 7.4%, demonstrating that the error in making a platelet count was no greater than that in making a red cell count. Counts made after standing 40 minutes on the counting chamber are as accurate as those made after 5 or 10 minutes, while shaking in a shaking machine for one hour, or storage for 24 hours in the icebox does not essentially alter the red cell count or the blood platelet count. The

* On account of its instability, heparin should be added to the Ringer's solution just before use and not to stock solutions. The formula of Ringer's solution used is: NaCl 9.0 gm., KCl 0.42 gm., $CaCl_2$ 0.24 gm., $NaHCO_3$ 0.10 gm., H_2O 1000 cc.

solution is evidently a favorable medium for the preservation of these blood constituents.

Fading and disintegration of the red blood cells and fragmentation and degeneration of the platelets, which are the usual sources of error in current direct methods of blood examinations, are essentially eliminated when Ringer-heparin solution is used as the diluting fluid. Platelets were not found to adhere to clean pipettes, and dirty pipettes influence the red even more than the platelet counts. By the time the red cell count is made, the platelets will be found to have settled sufficiently so that they may be counted immediately with the low power lens.

The use of this procedure for platelet counts which involves no change in the usual routine of red cell counts, has demonstrated its superiority over other methods in that accurate determinations are rapidly obtained within the time limits of a red count.

4902

The Fate of Foreign Sugars in the Blood Stream.

ELLA H. FISHBERG AND B. T. DOLIN.

From the Chemical Laboratory of Beth Israel Hospital, New York City.

The recent development (Somogyi[1]) of a rapid method for the estimation of foreign sugars in the blood in the presence of glucose throws new light on the chemical nature of the reducing substances circulating in the blood stream under normal and pathological conditions. Somogyi found that a simple substitution of a 10% (moist weight) yeast suspension for the distilled water used for laking and dilution in the Folin-Wu tungstic acid precipitation of the blood proteins results in the almost instantaneous total destruction of the glucose in the blood, leaving intact other sugars such as xylose, galactose and lactose and the various non-fermentable reducing substances in the blood.

The non-fermentable reducing substance of the blood was found to be remarkably constant in a series of patients not suffering from any metabolic disturbance and amounted to 28 ± 5 mg. per 100 cc. In another series of cases including uremia, diabetes, nephritis, this was found increased to as high as 90 mg. per 100 cc. Two patients in hypoglycemia from overdosage of insulin showed no reduction of

[1] Somogyi, M., *J. Biol. Chem.*, 1927, lxxv, 33.

the non-fermentable fraction. Pregnant women showed a high content of non-fermentable reducing substance.

Galactose and xylose introduced into the blood stream of rabbits disappeared at a rate proportional to the actual concentration of the sugar in the blood (Fishberg[2]). The mononuclear reaction formula $C = Ae^{-\alpha t}$ where C is the concentration of sugar in the blood, t is the time in minutes after injection, and α a constant gotten by elimination of A using any 2 separate experimental values of t, is found to apply to the rate of disappearance of foreign sugars injected into the blood stream.

If the kidney of the rabbit is damaged by the injection of uranium there is a distinct delay in the disappearance of the foreign sugar. On the other hand, if phloridzin is administered, the rate of disappearance of the sugar is much accelerated. If the rabbit is poisoned by phosphorus administered over a long period and shows definite fatty degeneration of the liver, there is a distinct retention of galactose in the blood stream.

In human beings galactose disappears more slowly from the blood of patients suffering from nephritis and diabetes. Especially xylose, which is excreted almost entirely through the kidney, shows a much slower rate of disappearance in nephritis, and the time of its disappearance from the blood stream is a good measure of the permeability of the kidney membrane.

4903

The Sedimentation Rate of the Erythrocytes in Chronic Arthritis.

M. H. DAWSON, RICHARD H. P. SIA,* AND R. H. BOOTS.

From the Arthritic Clinic† of the Presbyterian Hospital, New York.

Since the original observations of Fahraeus on the variation in the sedimentation rate of erythrocytes in health and disease the phenomenon has been studied in a wide variety of pathological conditions.

We have been interested in the reaction in only one class of cases —patients suffering from chronic multiple arthritis. Employing Westergren's[1] modification of Fahraeus' technique we have made

2 Fishberg, E. H., *J. Biol. Chem.*, 1930, lxxvi.

* On leave of absence from the Peiping Union Medical College, Peiping, China.

† Supported by the Faulkner Memorial Fund.

1 Westergren Alf, *Acta Medica Scandinavica*, 1920, liv, 247.

approximately 300 observations on 200 patients who sought treatment at the Arthritic Clinic of the Presbyterian Hospital.

Our results suggest that in this limited group of patients the determination of the sedimentation rate of the red blood cells is of distinct value. Our findings may be summarized as follows:

(1) In active cases of rheumatoid (chronic infectious) arthritis the sedimentation rate is greatly increased, usually attaining values above 40 mm. in one hour.

(2) In cases of osteo (hypertrophic) arthritis the sedimentation rate, while, as a rule, slightly elevated, rarely attains values greater than 30 mm. in one hour.

(3) Cases of so-called myositis, fibrositis and neuritis have almost invariably shown a normal sedimentation rate.

(4) In cases of rheumatoid (chronic infectious) arthritis the sedimentation rate parallels to an extraordinary degree the activity of the process.

The test as applied solely to arthritic patients is therefore of considerable value: (1) In differentiating the two great groups of arthritic patients—the rheumatoid or chronic infectious and the osteo or hypertrophic variety. (2) In differentiating between chronic multiple arthritis on the one hand and fibrositis, myositis and neuritis on the other hand. (3) In following the course of the disease and evaluating the results of therapeutic measures instituted.

4904

Effect of Feeding Specific Polysaccharide on Resistance to Pneumococcus.*

VICTOR ROSS.

From the Bureau of Laboratories, Department of Health, New York City.

The author has demonstrated that feeding the pneumococcus to white rats produces an increased resistance to the living organism. The acid killed intact cell,[1] the bile salt dissolved organism[2] and the mechanically disrupted pneumococcus[2] have been found effective as immunizing agents when administered by mouth. With the object of determining, if possible, the particular component of the pneu-

* The writer wishes to record his thanks to Mrs. Lawrance Harriman, who has kindly provided funds in aid of this work.

[1] Ross, Victor, *J. Lab. and Clin. Med.*, 1927, xii, 566.

[2] Ross, Victor, *J. Exp. Med.*, 1930, li, 585.

mococcus cell responsible for the observed effect, a degraded avirulent form of the pneumococcus, presumably producing no soluble carbohydrate,† was fed and it was shown[2] that comparatively little increased resistance was developed.

Recently, a carefully prepared sample of the specific polysaccharide of Type I pneumococcus was obtained through the courtesy of Dr. Harry Sobotka.‡ Experiments have been performed in which this material was dissolved in N/15 HCl, mixed with cracker meal, and fed to white rats. When subsequently examined these animals were found to possess an increased tolerance to intraperitoneal injection of the virulent organism.

Several facts had previously been shown to characterize the immunity produced by feeding the bacteria, either whole or dissolved, and offered a means by which one might determine to what extent the results obtained by feeding the specific polysaccharide resembled those gotten when the bacteria were administered. These were: (1) the immunity is produced by a single feeding, (2) it is present 48 hours following such ingestion, (3) in extent it is usually equivalent to 1,000 to 10,000 fatal doses (Type I), (4) when exhausted it can be made to reappear by a new feeding.

Of a group of 13 rats fed one dose of 0.5 mgm. of soluble polysaccharide (per rat), 5 survived when tested 48 hours later. Among them were animals which received 1, 10, 100 and 1,000 fatal doses of pneumococci. It has also been demonstrated that when the effect of a first immunization has worn off, a new feeding of the specific substance will cause it to reappear. It is thus seen that with regard to time of appearance, the need for but one dose, and renewability, the immunity produced by feeding the carbohydrate resembles that produced by the ingestion of the bacteria. The proportion of animals protected by a single administration of 0.5 mgm. of the former is, however, less than when the pneumococci from 5 cc. culture (containing less soluble specific substance) are used. It is possible

† The degraded avirulent pneumococcus failed to kill mice when 1 cc. of a 24 hour culture was injected. The organism was agglutinated by antipneumococcus serum regardless of type. No capsule could be demonstrated and the precipitin reaction carried out on the culture filtrate was negative.

‡ He found it to contain 5.0% nitrogen. The writer examined the sample for the presence of protein. The xanthroproteic and Millon tests were negative. The biuret was very faintly positive when done on a relatively large sample of dry material; with smaller quantities it was so faint as to cause a difference of opinion among observers. It was readily detectable in a dilution of 1 to 4,000,000. Dr. Frances Krasnow of the College of Physicians and Surgeons, Columbia University, kindly analyzed a sample and found by the Pregl micro method 41.7% C, 5.6% H.

that some alternation in the internal structure of the molecule of the polysaccharide takes place in the process of isolation, causing a partial loss of immunizing action. Greater uniformity follows the use of 2 or 3 such feedings. The degree of protection approaches that obtained with the organisms. The experiments indicate that the specific polysaccharide of pneumococcus, Type I, can act as an antigen when administered by mouth to rats. Schiemann and Caspar[3] found that mice become more resistant to pneumococcus following injection of a protein free solution prepared from pneumococci and containing the specific substance. It is still too early to say whether the specific substance is the only constituent of the pneumococcus which acts as an immunizing agent when the whole organism is ingested; the data, however, show that it plays a prominent part.

TABLE I.

Resistance of Rats Fed "Soluble Specific Substance," to Pneumococcus Type I.

Test done 48 hours after second of 2 consecutive daily feedings, each equal to 0.5 mgm. per rat. Test dose injected intraperitoneally in 0.2 cc.

C—Control. E—Treated Rat. D—died—days. S—Survived.

Weight	Dose	Result	Weight	Dose	Result
gm.	cc.		gm.	cc.	
C 90	10^{-9}	S	E 90	10^{-8}	D 4†
C 83	10^{-9}	S *	E 92	10^{-8}	S
C 97	10^{-8}	D 2	E 94	10^{-7}	S
C 95	10^{-8}	D 3	E 95	10^{-7}	D 4
C 98	10^{-7}	D 2	E 97	10^{-6}	S
C 98	10^{-7}	D 3	E 95	10^{-6}	D 2
C 105	10^{-6}	D 4	E 101	10^{-6}	S
C 117	10^{-5}	D 2	E 108	10^{-5}	S
			E 107	10^{-5}	S
			E 100	10^{-5}	D 2
			E 114	10^{-4}	D 2

* Sick, but recovered. † Ill before injection.

The accompanying table gives the results obtained in an experiment in which 0.5 mgm. of the specific polysaccharide was fed on each of 2 consecutive days.

[3] Schiemann, O., and Caspar, W., *Z. f. Hyg. u. Infektionskr.*, 1927, cviii, 220.

4905

Biochemical Studies of Bacterial Derivatives. X. Preparation of Human Tubercle Bacillus Protein MA-100.*

PETER MASUCCI AND KENNETH L. MC ALPINE.
(Introduced by F. M. Huntoon.)

From the Research Biological Laboratories of the H. K. Mulford Company, Glenolden, Pa.

In an effort to produce a potent tuberculin in a concentrated form and with the least possible chemical manipulation, Seibert[1] resorted to ultrafiltration, a method based on the conclusion from previous experiments that the active principle in tuberculin which is responsible for eliciting skin reactions in tuberculous guinea pigs is a water-soluble protein of the nature of an albumin. While this appears to be a promising method for concentrating the protein without incurring the possibility of denaturation, the end product is still complex since it retains all of the non-dialyzable constituent of the original filtrate among which are metaproteins, proteoses, and a large proportion of carbohydrate.

The aim of this investigation was to determine (1) whether the carbohydrate is linked up with the protein molecule in the form of a glucoprotein, and (2) if not, to prepare a protein fraction free from carbohydrate in order that its biological activity would not be masked by its presence. A method is outlined below for preparing such a fraction, to which we have given the designation of Human Tubercle Bacillus Protein MA-100 to conform with Johnson's scheme of nomenclature.

A culture of Tubercle bacilli (H-37) was grown on Long's synthetic medium and then filtered through a filter candle. To 11,000 cc. of this filtrate was added 7150 gm. $(NH_4)_2SO_4$. The precipitated protein soon rose to the top of the vessel and after standing several hours it was separated from the liquid by filtering through a Büchner funnel. The precipitate was purified by 8 successive reprecipitations. The precipitate from the last operation was dissolved in 1000 cc. distilled water, filtered through paper, and to the clear solution was added 30 cc. of Rimington's[2] salt buffer mixture (pH 4.7). Five volumes of 95% ethyl alcohol were added to the solution. A flocculent greyish-white precipitate formed, which gradually settled.

* This investigation was done in cooperation with the Medical Research Committee of the National Tuberculosis Association.

[1] Seibert, F. B., *J. Biol. Chem.*, 1928, lxxviii, 345.

[2] Rimington, Claude, *Biochem. J.*, 1929, xxiii, 428.

After standing over night the clear supernatant liquid was siphoned off, the precipitate thrown on a Büchner funnel and sucked dry. The precipitate which was readily removed from the paper was suspended in 320 cc. of distilled water, and just enough NaOH solution added to bring it to a pH of 7.4. The slight haziness of the liquid was removed by filtering through paper.

The clear yellow solution was adjusted to pH 4.7. A heavy flocculent precipitate formed which was allowed to settle. After standing several hours, the almost clear supernatant was siphoned off, and the precipitate was centrifuged. The precipitate was purified in this manner by three consecutive isoelectric precipitations. The final precipitate was dissolved in 320 cc. distilled water with the aid of a few drops of N/2 NaOH. Twenty cc. of this solution were reserved for test purposes. The remainder was reprecipitated once more at its isoelectric point, the precipitate was removed by centrifuging and then hydrolyzed with $Ba(OH)_2$ according to Rimington's[2] method in order to see whether any carbohydrate might be split off.

The supernatant from the fourth isoelectric precipitate gave only a weak Molisch test. Likewise one cc. of the purified Tubercle bacillus protein gave a faint positive Molisch. The purification process had removed almost completely the carbohydrate. The reducing properties of the carbohydrates in the various fractions were determined before and after hydrolysis by the Shaffer-Hartmann micro method.

TABLE I.
Distribution of Carbohydrate in Various Fractions Determined as Reducing Valued in Terms of Dextrose.

	Before Hydrolysis		After Hydrolysis	
	% Dextrose	Gm. Dextrose	% Dextrose	Gm. Dextrose
Original filtrate 11,000 cc.	0.144	15.8	0.485	53.3
8th ammonium sulphate precipitate dissolved in 1000 cc. water	0.005	0.05	0.390	3.9
Supernatants from 3 isoelectric precipitates made up to 1000 cc.	0.0000	0.0000	0.240	2.4
4th isoelectric ppt. hydrolyzed by the Rimington method—volume 94 cc.	0.17	0.016	0.028	0.026

An analysis of the data above shows, therefore, that 99.95% of the total reducing substances in the original filtrate was removed by this process. The carbohydrate, therefore, is not present in the form of a glucoprotein. The trace still present in the protein is

probably occluded and is not an integral part of the protein molecule.

The portion of the purified protein set aside for test purpose assayed 12.6 mgm. protein per cc. and gave a positive Biuret, Xanthoproteic, Millon and Molisch but a negative sulphur and Hopkins-Cole test. It contained a small amount of phosphorus. The protein solution was diluted and tested on tuberculous guinea-pigs and humans. It gave a positive typical skin reaction in doses as low as 0.00005 mgm. on humans. In comparison with O.T. and tuberculin made by Seibert's ultrafiltration method, this protein was relatively less toxic.

4906

The Relation of Vitamin D to Deposition of Calcium in Bone.*

H. C. SHERMAN AND H. K. STIEBELING.

From the Department of Chemistry, Columbia University, New York.

Quantitative studies in our laboratory[1] have shown that when the food of the growing animal (rat) includes a liberal allowance of vitamin D and a constant supply of phosphorus (0.42%), the calcium content of the bodies as determined at intervals during the period of growth is markedly influenced by the calcium content of the food, as varied in these experiments from 0.16 to 0.50% of calcium in the dry food mixture.

In the present experiments we find further, that in rats receiving a basal diet containing a generous allowance of calcium and phosphorus in good proportions (calcium, 0.74%; phosphorus, 0.58%) but with the vitamin D supply restricted practically to a bodily store acquired by 21 to 28 days of age, the growing body has at a given age about the same calcium content as has been acquired by similar animals which received 0.32% calcium, 0.42% phosphorus, and a liberal supply of vitamin D.

The calcium content of the femurs of rats which had been kept from the age of 21 or 28 days to the age of 56 days on the vitamin-D-deficient diet which we have previously described[2] is about twice as high as in otherwise similar rats fed the high-calcium rickets-producing diet of Steenbock (No. 2965) for the same length of time.

* Published as Contribution No. 624, Department of Chemistry, Columbia University.

[1] Booher, L. E., Dissertation, Columbia University, 1928.

[2] Sherman, H. C., and Stiebeling, H. K., *J. Biol. Chem.*, 1929, lxxxiii, 497.

Our rats have not had rickets according to the criterion of the "line" test, yet the deposition of calcium in their growing bones has been influenced by the vitamin D content of the food, even though the percentages of calcium and phosphorus in the dry food mixtures were favorable and uniform. Graded amounts of vitamin D supplied in the form of whole (summer) milk powder induced graded increments in calcification up to what appears to be the maximum potential level. In this particular series of experiments the favorable effect of the milk powder upon the normal calcification of the developing bone appears to be specifically due to the vitamin D which the milk contained and not to its mineral elements, because the largest amounts of milk powder fed to these rats did not significantly change either the ratio or the percentage of calcium or phosphorus in the total food, our basal diet being amply reenforced with a salt mixture approximating the composition of milk ash. Nor can the calcifying effect of the milk powder be attributed to fat (as such) or to lactose, for a few parallel experiments showed that the effect was not induced by the feeding of a mixture of milk ash, lactose, (commercial vegetable) fat, and purified casein, in the proportions found in milk solids.

From these new experiments, interpreted in the light of earlier work, it now seems clear that the interdependence of calcium, phosphorus, and vitamin D in bone development is such that under properly controlled conditions any one of the three may be made the limiting factor. In the work of Sherman and Pappenheimer[3] rats regularly became rachitic on a certain high-calcium, low-phosphorus diet and were regularly protected by the simple addition of a phosphate to their food. Here the diet was practically devoid of vitamin D but, the diet being such as to inhibit growth, the bodily store of vitamin D which the animals had previously acquired presumably remained as an available but subnormal supply during the experimental period. McCollum and his coworkers,[4] using diets similarly lacking in vitamin D and of constant phosphorus content, found marked differences to result from shifts in the calcium:phosphorus ratio. Our results also confirm and extend the finding of Steenbock and Black[5] and of Soames and Leigh-Clare[6] that vitamin D may be the limiting factor when the mineral content of the diet is good; and likewise

[3] Sherman, H. C., and Pappenheimer, A. M., *J. Exp. Med.*, 1921, xxxiv, 189.

[4] McCollum, E. V., Simmonds, N., Shipley, P. G., and Park, E. A., *Johns Hopkins Hosp. Bull.*, 1922, xxxiii, 296.

[5] Steenbock, H., and Black, A., *J. Biol. Chem.*, 1924, lxii, 275.

[6] Soames, K. M., and Leigh-Clare, J. C., *Biochem. J.*, 1928, xxii, 522.

the later work of Steenbock and his associates, of Fairhall, and of Sherman and MacLeod, which shows clearly that vitamin D can not take the place of the calcium needed for optimal bone development. Vitamin D appears to have influence upon the rate of normal calcification, even at levels well above those associated with rickets; but the supplying of vitamin D in abundance does not justify any lack of care in providing the growing organism with the liberal calcium intake which has also been shown to be essential to the optimal development of bone.

4907

Some Differences in Action Between Irradiated Ergosterol and Cod Liver Oil.

A. F. HESS, M. WEINSTOCK AND H. RIVKIN.

From the Department of Pathology, College of Physicians and Surgeons, Columbia University.

In a recent communication[1] it was reported that chickens require a much greater amount of irradiated ergosterol than its cod liver oil equivalent in order to protect them against leg-weakness. The potency of the irradiated ergosterol preparation used in these experiments has been standardized on rats on the basis of cod liver oil, as is the custom in the United States. In another communication[2] from this laboratory, it was shown that irradiated ergosterol, when given to rats in inadequate amounts, acted differently from cod liver oil, in that it raised the inorganic phosphorus of the blood to its normal concentration without healing the rachitic lesion.

The action of these two specific antirachitic agents is likewise not identical in relation to infantile rickets. This winter, in the course of an extended series of observations, it was found that 20 drops of a standard preparation of irradiated ergosterol (viosterol) in some instances failed to fully protect infants from rickets, although the inorganic phosphorus concentration of the blood was maintained. According to the standard biological test, 20 drops of this preparation is equivalent to 10 teaspoonfuls of cod liver oil. In one in-

[1] Hess, A. F., and Supplee, G. C., PROC. SOC. EXP. BIOL. AND MED., 1930, xxvii, 609.

[2] Hess, A. F., Lewis, J. M., and Rivkin, H., PROC. SOC. EXP. BIOL. AND MED., 1930, xxvii, 611.

stance in which mild rickets developed although this amount of irradiated ergosterol had been given daily, 6 teaspoonfuls of cod liver oil subsequently brought about healing in a few weeks as demonstrated by radiographs.. In other words, 6 teaspoonfuls of cod liver oil brought about cure where 20 drops of irradiated ergosterol (supposed to be the equivalent of 10 teaspoonfuls of cod liver oil) failed to afford complete protection. Such a result does not mean that the antirachitic action of irradiated ergosterol is weak or unreliable, for undoubtedly complete protection or cure would have been brought about by a larger dosage. Cod liver oil not infrequently fails to fully protect when given to infants in the customary dose of 3 teaspoonfuls a day. An observation of this kind indicates rather that we have a faulty conception of the relative potencies and dosages of the two specifics.

These inconsistencies may be due to the fact that irradiated ergosterol contains a phosphate-raising factor in addition to its well recognized antirachitic fraction. However this may be, it would seem evident that the present method of standardizing irradiated ergosterol is unsound from a clinical point of view, as it is based on the assumption that the relative antirachitic potency of cod liver oil and of irradiated ergosterol is the same in the rat and the infant, and that the units are interchangeable. Instead of an indirect method which computes activity on the basis of "cod liver oil units", it would be better to determine the number of protective or curative "rat units" and to use this direct determination as the basis of standardization.

4908

Inorganic Constituents of Blood and Urine in Dogs with Pancreatic Fistula.

T. F. ZUCKER, M. GUTMAN-NEWBURGER AND B. N. BERG.

From the Department of Pathology, College of Physicians and Surgeons, Columbia University.

Several studies on the effects of drainage of pancreatic juice have appeared during the last few years, verifying and extending Pawlow's original observations. Elman and McCaughan[1] demonstrated that drainage of the pancreatic juice through a fistula will cause

[1] Elman and McCaughan, *J. Exp. Med.*, 1927, xlv, 569.

death in a short time. Gamble and McIver[2] showed that the symptoms and death were due principally to a loss of fixed base and chloride, which resulted in dehydration of the blood. The fixed base and chloride of the plasma progressively diminished and water was lost while the plasma protein increased quite markedly. Hartman and Elman[3] confirmed the findings of Gamble and McIver and alleviated the symptoms and prolonged life by the injection of salt solutions.

Our own work on pancreatic fistulas, having initially a different objective, dates back to 1927. Observations were made on total base of serum, pancreatic juice and urine (methods of Stadie and Ross[4]), specific conductivity of serum (Kohlrausch) serum protein (refractometer), chloride and bicarbonate. Chlorides and creatinin were also determined in urine. Depression of freezing point of the pancreatic juice was also done in a large number of samples.

The results were as follows: A series of over 20 dogs was studied. The dogs on a diet containing salt showed a mean survival time of 15 days, or else failed to show any evidence of dehydration. The dogs on a diet without salt showed a survival time of 8 or 9 days. All dogs injected with NaCl or NaCl + $NaHCO_3$ after dehydration had begun, showed a response to treatment. The initial dehydration could be entirely overcome by the injection of electrolyte in quantities a little more than equivalent to the juice secreted. In dog 29, the rise and fall of total base in the serum was followed at intervals of a few days throughout 53 days of intermittent injections. When the volume of isotonic saline injected was considerably larger than the juice secreted, the serum protein fell distinctly below normal, but the total base never rose above normal.

The initial total base was not affected by diet, but the susceptibility to dehydration apparently was. This would suggest that with constant serum base, other tissues may store or lose base. Previous feeding on a salt-containing diet raises the animal's resistance to dehydration and a salt-free diet lowers it. It also appears that, while normal animals do not show much change in serum base after injection of salt an animal with pancreatic fistula is markedly influenced in this regard.

We find a good agreement between the results of total base determination by the method of Stadie and Ross and the specific conductivity corrected for protein, the ratio in a given animal being

[2] Gamble and McIver, *J. Exp. Med.*, 1928, xlviii, 849.

[3] Hartman and Elman, *J. Exp. Med.*, 1929, l, 387; Elman and Hartman, *Arch. Surg.*, 1930, xx, 333.

[4] Stadie and Ross, *J. Biol. Chem.*, 1925, lxv, 737.

quite constant (within 2½%, which is about the limit of error of method).

As a check and an alternative method for total base in pancreatic juice we have used the freezing point depression and here also the ratio Δ/T.B. ran very nearly constant.

The total base content of the pancreatic juice is very constant from animal to animal, being a few milli-equivalents higher than that of the plasma, and during depletion of plasma base, decreases proportionately. Under these same conditions, the urine is decreased very markedly in both volume and total base. As a secreting gland, therefore, the pancreas takes precedence over the kidney in its lien on both water and base, as may be seen from metabolism experiment on dog 48. This dog was on a diet containing about 30.7 milli-equivalents of total base per day. Before operation a 24-hour urine contained 29.0 milli-equivalents. Five days after operation the urine contained 14.2 milli-equivalents of base, while 39.5 milli-equivalents were lost through juice secretion. After 3 days more, pancreatic secretion had practically ceased owing to a plugged canula, and 24.5 milli-equivalents of base were found in urine.

The symptoms observed, although due to loss of electrolyte, are not dependent simply on the electrolyte level in the plasma. The rate of change is a large factor. Under conditions of slow dehydration the animal may, without any symptoms, attain a low level of electrolyte, which would be fatal if the change were a rapid one.

4909

The Killing of Moulds by an Ordinary Electric Bulb.*

BASILE J. LUYET. (Introduced by Ross G. Harrison.)

From the Osborn Zoological Laboratory, Yale University.

Darkness provides optimum condition for the growth of moulds, and ultraviolet rays exert a rapid and violent fungicidal effect on them, but one does not usually suspect the extreme sensitiveness of the *Mucoraceae* to the feeble light of an ordinary electric lamp. Our

* This work was done under a Seessell fellowship grant. The ultraviolet lamp was provided by Hanovia Company, through the Committee of the National Research Council on the Effects of Radiation on Living Organisms. *Mucoraceae* pure cultures were kindly supplied by Dr. A. F. Blakeslee, of the Carnegie Institution.

attention has been drawn to this point by the fact that *Mucoraceae* cultures, while on our experimenting table, awaiting examination, have been killed by the light of the microscope lamp.

Spores of *Rhizopus nigricans,* in suspension in sterilized water, were spread, by means of a soft brush, on the surface of agarized Coon's medium,† sterilized and distributed in a series of Petri dishes. These cultures were irradiated, through the glass covers of the dishes, by the light of an ordinary bulb, labeled: "Westinghouse, Mazda, 60 W, 115 V." and working on direct current. There was a distance of 5 cm. between the cultures and the luminous filament. An electric fan cooled the dishes and excluded the influence of heat.

Irradiations were made as follows: 1. Half an hour after the inoculation with the spores, that is, before any germination. 2. At the end of 15 hours, when the sprouts were some mm. long. 3. At the end of 30 hours, when the length of the mycelium exceeded 1 cm. 4. At the end of 40 hours, when the sporangia have developed. (These stages of growth are attained in the indicated intervals at a temperature of 22°C.)

Under these conditions we have observed, in 15 different experiments, that 20 to 30 minutes exposure destroyed the spores, that 5 to 8 minutes killed the mycelium of 15 to 30 hours, but that 5 hours irradiation were not sufficient to cause the death of sporangia.

This method of destroying moulds will possibly be of practical application. But, while light can thus be used as an excellent preventive against the sprouting of *Mucoraceae,* the resistance of sporangia to irradiation excludes its use as a curative agent after the mustiness has already developed.

In other experiments, variously colored screens were interposed between the bulb and the culture and it has been possible, despite these rather dark screens, to destroy the mycelium of the same moulds by exposures varying in duration from 5 to 30 minutes. These observations are but preliminary trials of a more complete study, now in progress, of the minimal lethal dose for each wavelength.

Coextensive with the experiments just described, similar ones were performed with ultraviolet light. We used a mercury arc lamp, working with 4 amperes and 60 volts, on direct current. The duration of exposure varied from 5 to 120 seconds. The distance between the lamp and the culture was 30 cm. The dishes were without cover during the irradiation. Observations were made on single

† $MgSO_4$: gr. 0.5; KH_2PO_3 : gr. 1.36; Asparagine : gr. 0.26; Maltose : gr. 3.6; water : gr. 1000.

individuals. After locating a germinating spore, under the microscope, by a small dot of ink placed adjacent to it, we followed its growth by a series of camera lucida drawings, all or half of each Petri dish having been exposed to the light. One hundred and seven experiments have thus been made, 42 on spores, 54 on mycelia and 11 on sporangia.

Under these conditions, 45 seconds irradiation brought about death of the spores, 15 to 45 seconds produced a delay in their germination and growth, and 10 seconds were absolutely ineffective. The same effects were produced on mycelia by 25, 5 to 25, and 4 seconds exposure, respectively. Mature sporangia, removed from the sporangiophores, have been irradiated singly, on a glass slide, for 3 hours. After exposure, germination proceeded normally.

The slowing up in growth, noticed after irradiation of spores and mycelium, is an increasing function of the period of exposure. We observed, in particular, that the greater the exposure the greater was the delay in appearance of sporangia. The radiosensitivity of mycelia did not vary, in these experiments, with their age.

The difference in sensitiveness to ultraviolet light between spores, mycelium and sporangia, already described for *Mucor hiemalis* in a previous notice,[1] has thus been confirmed here in *Rhizopus nigricans,* and extended to the wave-lengths of visible light.‡

4910

A Comparative Study of the Total Red Counts of Wild and Liver-fed Trout.

CHRISTIANNA SMITH. (Introduced by A. E. Adams.)

From the Walter H. Merriam Laboratory, Department of Zoology, Mount Holyoke College, South Hadley, Mass.

Because of the use of liver as a food for young trout, it was suggested to the writer that a comparative study of the blood of wild and liver-fed trout might show the reaction of normal and growing animals to liver. Although the data are limited, the results indicate an increase in the total red counts of liver-fed trout.

Method. It is well known that fish blood is difficult to work with

[1] Luyet, B., *C. R. Soc. Phys. Hist. Nat. Genêve,* xlvi, 2, 107.

‡ We express our gratitude to Professor R. G. Harrison for his suggestions and his kind assistance.

because it clots quickly, is easily contaminated with mucus, is relatively limited in quantity in small fish, and may change if the fish are out of running water for any length of time. Through the courtesy of the Massachusetts Department of Conservation, Division of Fisheries and Game, blood was obtained from wild trout at the Palmer Hatchery and from liver-fed trout at the Sunderland Hatchery. Haemoglobin determinations were made at the hatcheries but the pipettes for the total counts were brought back to the laboratory. The blood both for haemoglobin determinations and for total counts was taken directly from the ventricle or the sinus venosus into the pipettes.

Total Red Counts. Table I and Fig. 1 give the results of counts made on 11 wild trout and 17 liver-fed trout. Although the lengths of the two kinds of trout are about the same, those which were fed

TABLE I.
Wild Trout (approximately 1 year old).

Date	Number	Sex	Length in inches	Total Red per cmm.	Hb Tallquist
					%
4/14/28	2			1,800,000	
	3			1,670,000	
	4			1,440,000	
	5			2,080,000	
	6			3,120,000	
4/21/28	7		6¾	2,240,000	50
	8	Male	7¼	1,810,000	40
	9	Female	7½	2,060,000	
	10	Male	7¾	1,785,000	
	11	Female	5¾	1,930,000	35
	12	Male	5¾	2,280,000	30
			Liver-fed Trout.		
5/12/28	13	Male	7¾	2,366,000	
	14	"	7⅛	2,070,000	
	15	Female	7½	2,300,000	50
	16	"	7¼	2,647,000	50
	17	Male	8¼	2,595,000	50
5/19/28	18	Female	7½	3,355,000	40
	19	Male	7¾	2,025,000	60
	20	"	8	2,405,000	60
	21	"	7½	2,140,000	60
	22			2,455,000	
	23	Male	7¾	2,335,000	50
5/24/28	24	"	8	2,895,000	
	25	"	7½	2,665,000	60
	26	Female	7	2,360,000	60
	27	"	7	2,595,000	50
	28	Male	7⅞	2,290,000	50
	29	"	7½	2,675,000	50

In most cases 4 drops were counted from each pipette to obtain the total number of red cells.

liver possessed much more fat. While 6 of the 11 wild trout have counts less than 2,000,000, all of the 17 liver-fed have total numbers above that value. There appears to be no difference due to sex. The one count of 3,000,000 among the wild trout was obtained from a fish which had been in a bucket of water for one and a half hours before the blood was taken and was consequently very inactive. The mean of the counts for the wild trout is 2,020,000 ± 89,000; for the liver-fed, 2,480,000 ± 54,000. As the difference between the 2 means is 4 times its probable error, it would seem that the difference between the total counts of the wild and liver-fed trout may be significant.

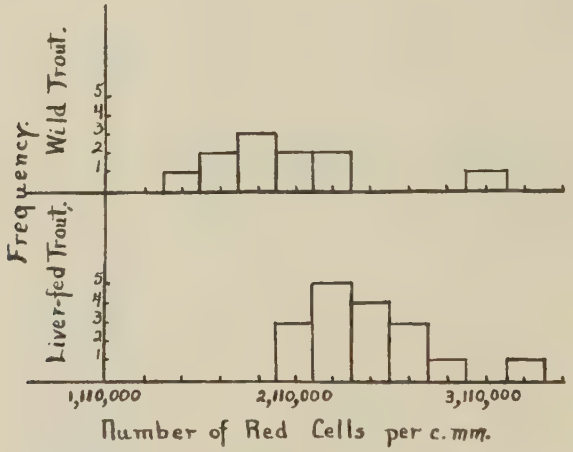


FIG. 1.
Diagram of frequency distribution of red-blood-cell counts in 11 wild trout and 17 liver-fed trout.

Haemoglobin Determinations. The results of this part of the work are quite unsatisfactory. In the majority of cases determinations were made with the Dubosc colorimeter and the Tallquist scale. The readings made with the Dubosc colorimeter have been discarded because, although an attempt was always made to use northern light, the lighting was not uniform, the yellow filter was not accurate, and there were other errors in adjustment. The values obtained by the use of the Tallquist scale give a general idea of the reactions of the liver-fed trout.

Discussion. A recent paper by McCay[1] gives some preliminary counts on normal fish from different genera and on individuals from the same species. The mean of the counts from the 9 genera is

[1] McCay, C. M., "A Biological Survey of the Erie-Niagara System." 1928. State of New York, Conservation Department, 1929. 140.

1,996,000 ± 127,000. The figures given for 5 carp, same species, are 1,600,000, 1,400,000, 1,700,000, 1,500,000, 1,800,000; for 3 bullheads, 2,800,000, 1,300,000, 1,300,000. From these few data and the results obtained in this study for wild trout, the conclusion would seem to be valid that the total red counts of the liver-fed trout tend to be higher. It seems likely that the liver diet is responsible for these high counts, but other factors may be influential. These factors may be differences in food, other than or in addition to the liver, which have also increased the deposition of fat in the liver-fed trout or they may be connected with the water content of the streams, the relation of which to the physiology of fish is little understood.

4911

Vasomotor Control of the Liver Circulation.

FRED R. GRIFFITH, JR., AND F. E. EMERY.

From the Department of Physiology, University of Buffalo, Buffalo, N. Y.

Our present knowledge of the vasomotor control of the liver circulation is curiously inadequate. In due time the evidence for this conclusion will be reviewed in detail; now we wish merely to record a summary of the results which have been obtained during the last couple of years in a study of this problem, using cats under chloralose anesthesia and the liver plethysmograph previously referred to.[1, 2]

The peripheral vagus has no effect on liver volume; we have stimulated it in the neck (after denervating the heart according to Cannon's method), below the heart in the thorax and after emerging through the diaphragm.

The postganglionic fibers of the hepatic plexus constrict not only the terminals of the hepatic artery but also those of the portal vein.

The preganglionic fibers of the splanchnic (left) have exactly the same effect on liver volume, either by way of the artery or portal vein, as stimulation of the postganglionic fibers in the hepatic plexus.

Reflex pressor responses are accompanied by decreased liver volume; depressor reflexes produce dilation in the liver. If, however, the liver is denervated by cutting the fibers of the hepatic plexus, its volume then follows passively the general blood pressure.

During the generalized vasomotor activity induced by asphyxia

[1] Griffith, York and Zachmys, PROC. SOC. EXP. BIOL. AND MED., 1928, xxv, 399.
[2] Griffith and Emery, *Ibid.*, 1929, xxvi, 628.

(rebreathing the air in a small balloon) the liver constricts powerfully during the rise of general blood pressure and remains constricted even as the heart begins to fail. If it is denervated, however, it dilates for a time as the general blood pressure is rising; but before this has reached its maximum the liver often begins to constrict maximally. This delayed constriction may be prevented by removal of the adrenals; then the denervated liver volume passively follows the general blood pressure throughout the course of the asphyxia.

4912

Evaluation of X-Ray Evidence as Criteria of Intestinal Obstruction.

OWEN H. WANGENSTEEN AND FRANCIS W. LYNCH.

From the Department of Surgery, University of Minnesota.

Late diagnoses in simple obstruction of the bowel are due in large measure to the absence of local physical findings which help in other abdominal disasters to corroborate the suspicion that a surgical emergency obtains. The usual textbook description of the physical findings in intestinal obstruction is an ante mortem picture. Regurgitant vomiting and abdominal distension are heralds of death and not physical findings significant of the presence of acute bowel obstruction.

The employment of the X-ray to detect bowel obstruction was suggested about 20 years ago, but it is not widely used for this purpose today and several recent writers state that the employment of X-rays is of no value in the diagnosis of obstruction. In this study an attempt has been made to evaluate the X-ray criteria in the early recognition of bowel obstruction.

Normally gas exists throughout the entire intestinal tract, but when an X-ray film is made of the abdomen, gas is discernible only in the stomach and colon, and especially at the flexures. Its intimate admixture with fluid in the small intestine precludes its demonstration on the X-ray film, though tiny bubbles are constantly present at least in portions of the small intestine. The accumulation of the gas in the small intestine is therefore proof of the existence of delay in transit of the content through the small intestine and usually means intestinal stasis.

In this study simple obstruction was established in 20 dogs; 16

of these were complete, the bowel being severed and the ends inverted. In the other 4, partial obstructions were established by ligating the bowel with gauze. Seventeen dogs were obstructed in the lower one-third of the small intestine and 3 were in the sigmoid. One of the latter animals lived 50 days. In 2 dogs X-ray observations were made following the establishment of strangulation obstruction accomplished by a ligature of an intestinal loop 3 feet in length together with its mesentery. All of the obstructions were done aseptically; the simple obstructions were made under ether anesthesia, and the strangulation obstructions were done under procaine infiltration of the abdominal wall reinforced by a preliminary injection of morphine.

In 3 animals after establishing obstructions low in the ileum gastrostomies were made and barium given post-operatively in an attempt to determine the time at which the barium would reach the site of intestinal occlusion. The animals were X-rayed frequently post-operatively and daily measurements of the abdominal circumference were made until the time of death.

In some instances saline was administered subcutaneously—400 cc. of 1% solution being given daily. In a few instances perforated lead shot were fastened to the bowel proximal to the obstruction such that an accumulation of gas in the intestine could easily be followed and its location easily interpreted. In a group of 7 dogs with simple obstruction, plates were taken at intervals of 1 to 2 hours for the first 9 hours after the obstruction, with the point in mind of determining when gaseous distension of the intestine occurred.

In summarizing briefly the results of these experiments, we demonstrated that the X-ray film without the employment of the contrast medium is a reliable method of determining a block in the intestine. Within 4 or 5 hours after intestinal occlusion there was definite evidence of gaseous distension in the loops of the small intestine. After 20-24 hours the distension of the intestine proximal to the point of obstruction was fairly general even though clinical distension was usually not obtained. A measurable increase in the circumference of the abdomen was usually noted only about 24 hours before death. It was an interesting observation in these experiments that in simple obstruction fluid does accumulate above the point of intestinal occlusion though not in the same measure as observed in the human. When saline was administered subcutaneously, however, fluid levels or mirrors were obtained when the dogs were rayed in the standing posture. In one instance only 35 cc. of fluid was obtained from the intestine proximal to the obstruction at death,

but definite fluid levels could be seen in the X-ray film. In the 2 dogs in which strangulation obstructions were established gaseous distension of the proximal loops was noted on the X-ray film, but the strangulated segments failed to exhibit dilatation. A report of further X-ray studies on strangulation obstruction will be made subsequently.

In the instances in which barium was given through a gastrostomy tube an interval of 20 hours was usually necessary before the barium reached the site of intestinal block. The employment of contrast media to determine the presence of bowel occlusion is to be condemned, but flat plates of the abdomen constitute a measure of real value in detecting intestinal stasis. The stethoscope will serve to differentiate whether the obstruction is mechanical or inhibitive. The former exhibits loud intestinal noises; in the latter the abdomen is silent.

4913

Motor Activity of the Distal Bowel in Intestinal Obstruction: Comparison with the Obstructed and Normal.

HERBERT A. CARLSON AND OWEN H. WANGENSTEEN.

From the Department of Surgery, University of Minnesota.

The bowel distal to the point of obstruction is normal anatomically, whereas the proximal bowel exhibits anatomical changes. Alvarez[1] states that most of the motor activities of the stomach and bowel are brought about and regulated largely by the internal pressure due to the presence of food or gas, on which basis an eventual condition of inactivity in the bowel distal to a complete obstruction would appear to be understandable. But the distal bowel has been found to evacuate enemas and transport barium in approximately normal time. In this study an attempt has been made to study the motor activity of the distal segment of the bowel in the presence of simple obstruction. There were 22 dogs obstructed, the point of severance being usually 3 to 5 feet above the ileo-caecal valve. The proximal end was turned in and the distal end anchored to the skin in 8 dogs. In 14 others both ends were inverted.

Small rubber balloons were inserted into the bowel and the contractions of the bowel recorded upon a smoked drum by means of a

[1] Alvarez, W. C., "The Mechanics of the Digestive Tract." Paul B. Hoeber, New York. 1928. 24.

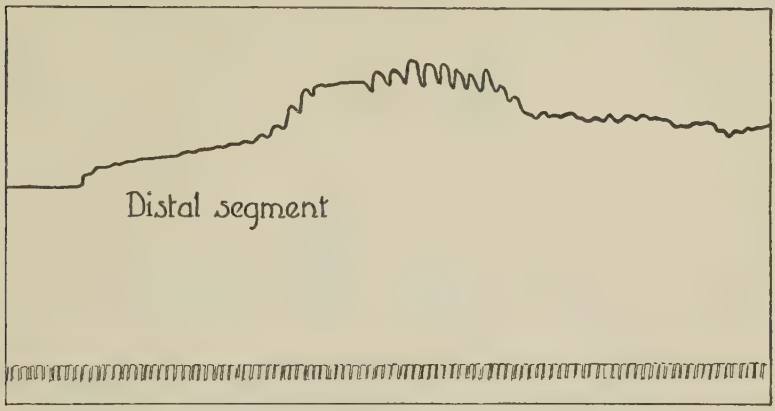


FIG. 1. Dog obstructed 30 hours. Tracing of distal segment.

water manometer. From the distal segment tracings could be obtained through the enterostomy without the employment of anesthesia.

Tracings in the normal bowel show rhythmic contractions occurring about 10 to 15 times per minute. The height of the contraction varies to such an extent in different animals and at different times in the same animals that no numerical average can be adopted for quantitative comparisons. However, tracings of the distal loop

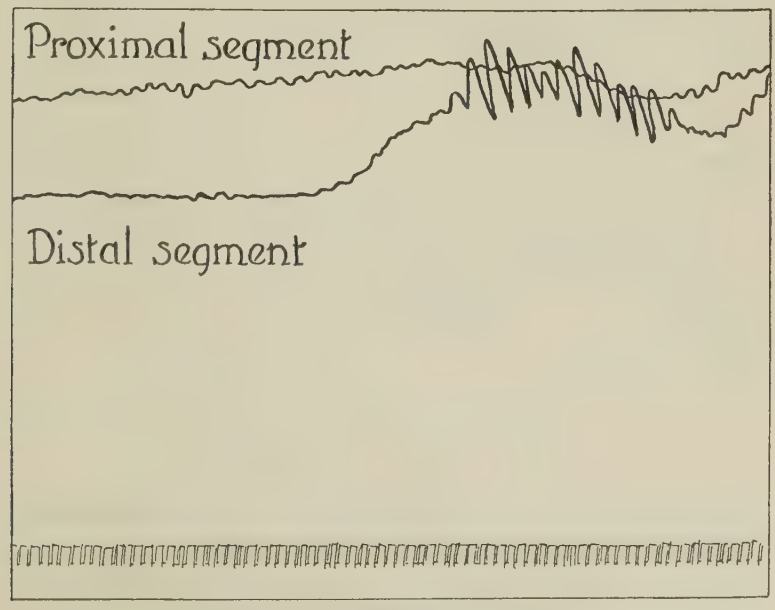


FIG. 2. Dog obstructed 5 hours.

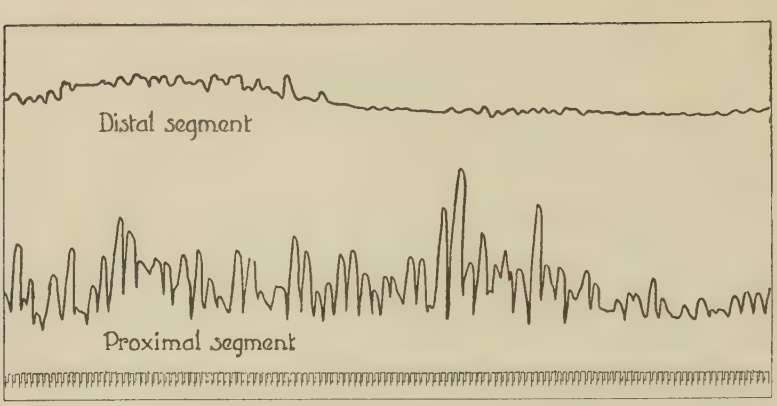


FIG. 3. Dog obstructed 7 days.

resemble closely tracings of the normal bowel studied under similar conditions.

In a comparison of the activity of the proximal and distal loops recorded simultaneously on the same tracing in 13 experiments the greater activity occurred in the proximal loop 5 times, in the distal loop 7 times and in one case (Fig. 2) the greater activity occurred first in the proximal and later in the distal segment. In this small series of observations, therefore, the greater activity was found more commonly in the unobstructed bowel.

On the other hand, comparison of the maximum contractions of the various bowel loops indicate that the obstructed loop shows the

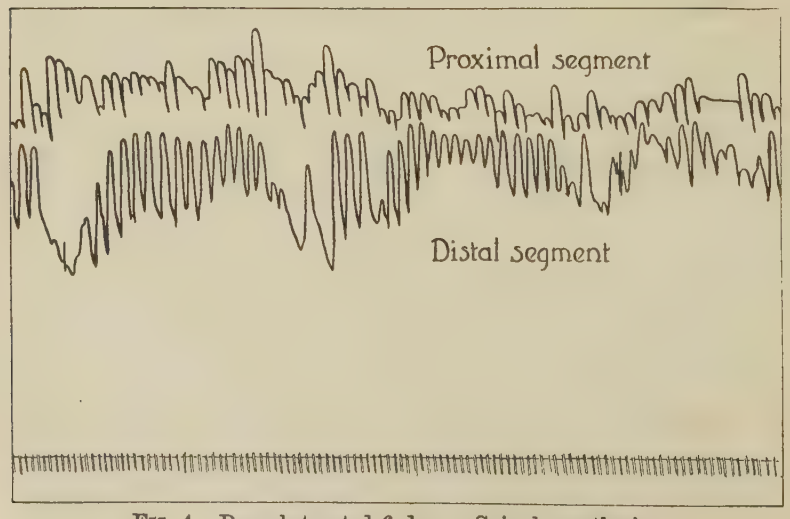


FIG. 4. Dog obstructed 6 days. Spinal anesthesia.

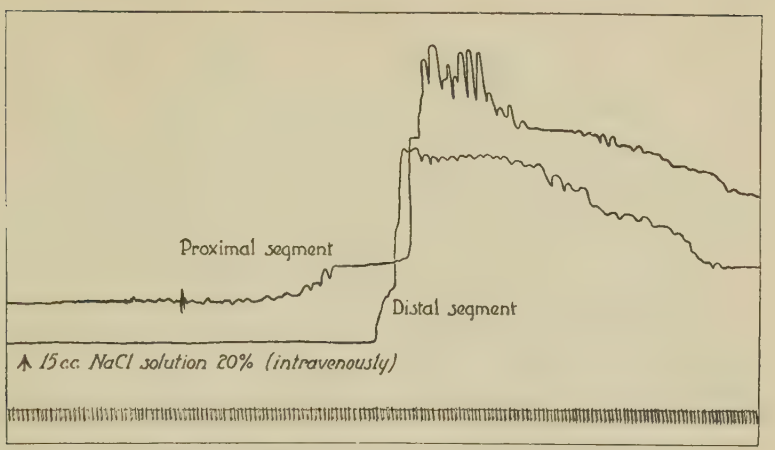


FIG. 5. Effect of hypertonic salt solution.

greatest maximum contractions; the normal bowel is intermediate and the maximal contraction of the loop below the obstruction does not attain that of the other two.

The differences in the maximum contractions, if they are significant at all, are undoubtedly dependent upon the intra-intestinal pressure, for the sustained intra-intestinal pressure in the obstructed bowel is about twice that of the normal and during activity may arise to 10-15 times the normal pressure. (Owings and associates.[3])

Figures 1-4 illustrate variations in the relative activity of the bowel segments in different tracings. The great activity of the bowel segment obstructed for 7 days (Fig. 3) is unusual and is in disagreement with the findings of Owings and his associates, who observed a decrease of pressure and activity after the fifth day. The greater incidence of peritonitis in the pressure experiments may

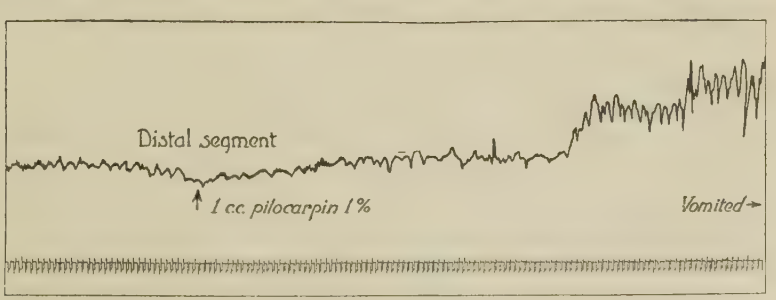


FIG. 6. Effect of pilocarpin.

[3] Owings, J. C., McIntosh, C. A., Stone, H. B., and Weinberg, J. A., *Arch. Surg.*, 1928, xvii, 507.

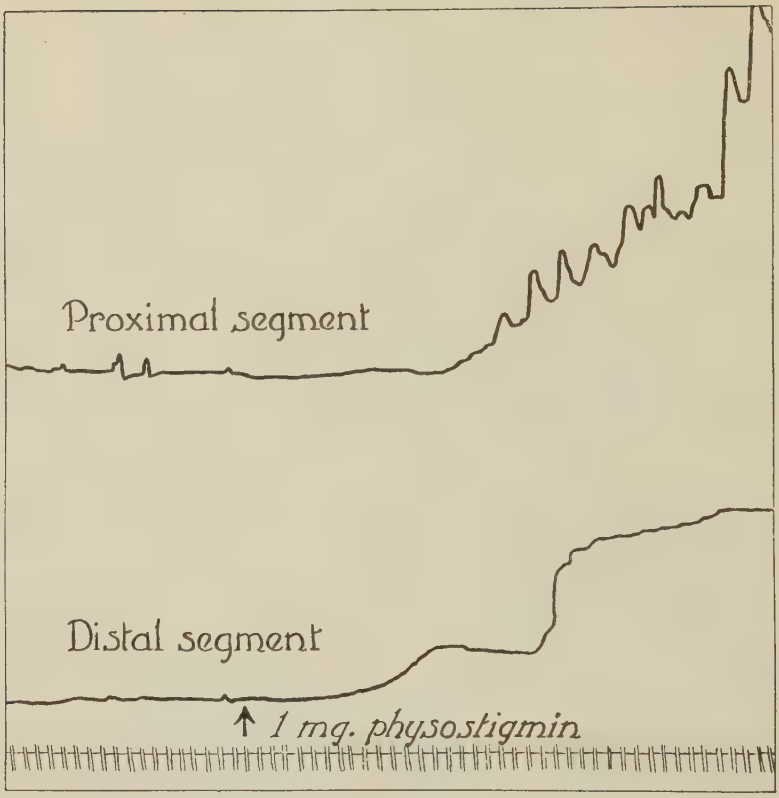


FIG. 7. Effect of physostigmin.

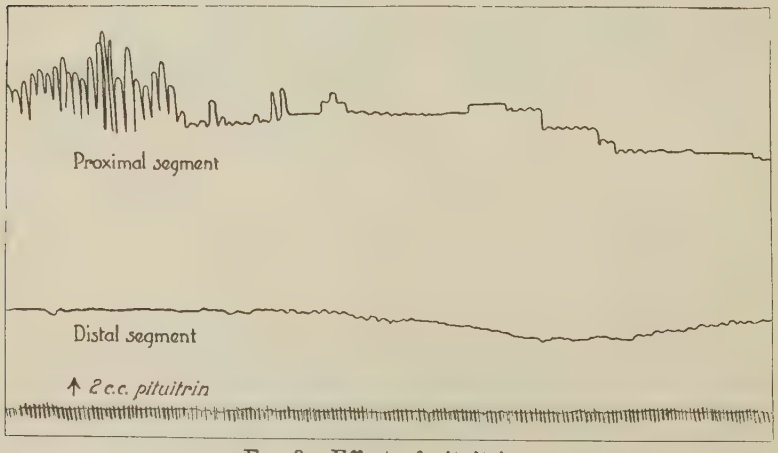


FIG. 8. Effect of pituitrin.

play a rôle although Hotz[4] believed that the decreased function of the bowel in peritonitis was due to gaseous distension.

The action of pituitrin (Fig. 8) occasioned not a little surprise. Doses of 0.5 cc. to 2 cc. of surgical pituitrin intra-muscularly caused no response at all or a depression of the intestinal movements. A dose of 1 cc. intravenously resulted in a reduction of the activity and decrease of the tone. The greater effect appeared to be on the segment of bowel which at the time showed the greater activity regardless of whether this was the obstructed or the unobstructed segment. In some cases the period of inactivity was followed by one of activity but in no case did the secondary activity exceed that prior to the administration of the drug. The same results were obtained in the study of the effect of pituitrin on the normal bowel. Our findings agree with those of McIntosh and Owings[5] who also point out that the literature relative to the effect of pituitrin on the bowel is in disagreement. Further work is needed to clarify the action of this drug on the intestine of man and laboratory animals under normal and pathological conditions.

In conclusion it may be said that the segment of bowel distal to a complete obstruction has a capacity for normal physiological activity and responds to chemical stimuli in a normal manner. This segment of the bowel is normal physiologically as well as anatomically.

4914

Electrocardiographic Diagnosis of the Artery Occluded in Cardiac Infarction.

WERNER J. ROSE AND FRANK MEYERS. (Introduced by B. D. Bowen.)

From the Buffalo General Hospital and the School of Medicine, University of Buffalo.

Changes in the electrocardiogram due to cardiac infarction have been noted by many observers, and reports of clinical cases give us our most valuable information because the coronary system in the common laboratory animals does not parallel the human coronary circulation. Thus it is not possible to study the effect of infarction in various parts of the animal heart by laboratory methods and apply these findings to human cases.

[4] Hotz, G., *Mitteil. a. d. Grenzgeb. d. Med. u. Chir.*, 1909, xx, 257.

[5] McIntosh, C. A., and Owings, J. C., *Arch. Surg.*, 1928, xvii, 996.

Parkinson and Bedford[1] noted that in human cases of cardiac infarction they obtained 2 distinct types of curves. They call a curve T_1 type when the R-T alteration occurs in leads I and II, and T_3 type when the R-T alteration occurs in leads II and III. In some of their cases they noted that with one attack they obtained a certain type and during a subsequent attack, the other type.

Barnes and Whitten[2] accurately studied the site of infarction associated with the 2 types of T wave alteration and concluded that infarction in the anterior left ventricle, which is always due to occlusion of the left coronary artery, is commonly associated with the T_1 type of curve; and infarction in the posterior wall of the left ventricle, which is mainly supplied by the right coronary artery as demonstrated by Gross, is associated with the T_3 type of curve.

Observations of the writers may serve to verify these conclusions. The cases presented below demonstrate our findings.

Figure 1 shows a tracing, (T_1 type) encountered in a typical case of cardiac infarction occurring in the anterior wall of the left ventricle due to occlusion of the descending branch of the left coronary artery.

Figure 2 is a tracing recently encountered in which the changes seen in the electrocardiogram are in leads II and III (T_3 type) and in view of the previous tracing and the survey of Barnes and Whitten, a diagnosis of infarction in the posterior left ventricle due to occlusion of the right coronary artery was ventured. Autopsy proved this assumption correct.

The heart was removed one hour after death and injected according to the method of Hill.[3] Figure 4 shows that the left coronary artery (L) fills well.

The right coronary artery (marked by lead bar) fails to fill as far as the posterior descending artery. The main stem of the right artery is faintly visible by X-ray due to the large calcium deposit in the sclerotic artery.

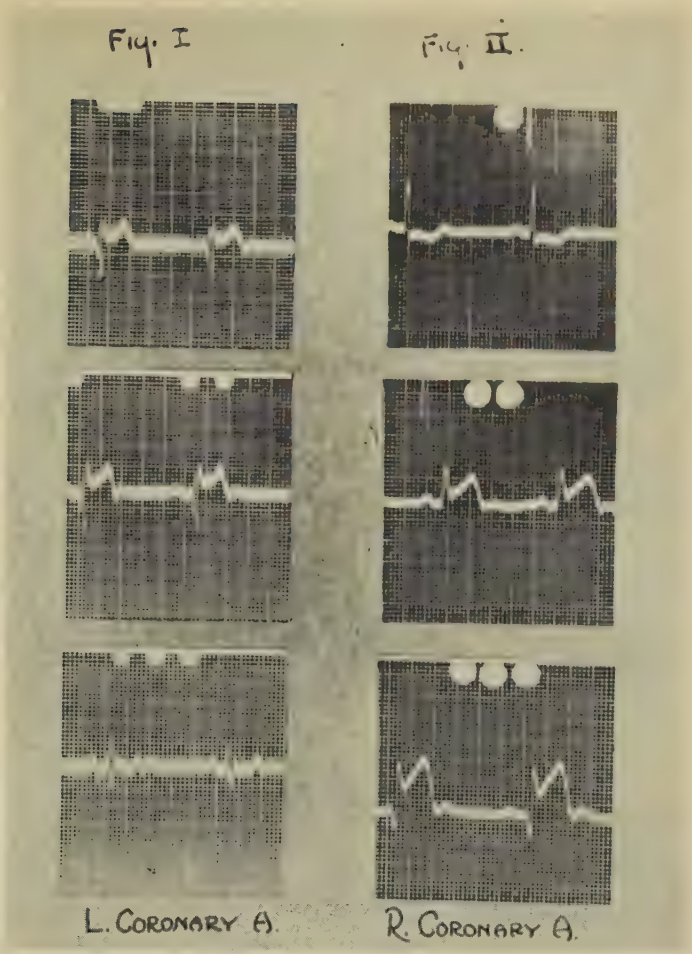
Conclusions. 1. Infarction of the anterior wall of the left ventricle is always associated with occlusion of the left coronary artery. Infarction of the posterior wall of the left ventricle, in the absence of anomalies, is almost always associated with occlusion of the right coronary artery.

---

[1] Parkinson and Bedford, *Heart*, 1928, xiv, No. 3.

[2] Barnes and Whitten, *Am. Heart J.*, 1929, v, No. 2. Gross-Monograph on Coronary Circulation.

[3] Hill, E. C., *Johns Hopkins Bull.*, 1929, xliv, 3.



FIGS. 1 and 2.

2. When the infarction involves the anterior wall, the elevation of the R-T segment of the electrocardiogram is in leads I and II, and conversely when in the posterior wall, the elevation of the R-T segment is in leads II and III.

3. Knowing the rôle of the right coronary artery in the supply of the posterior wall of the left ventricle, when we encounter a case of coronary occlusion in which the electrocardiogram shows R-T elevation in leads II and III, we can say with reasonable certainty that the occlusion is in the right coronary artery provided there is no anomaly of that artery.

4. It has been demonstrated that the site of infarction and artery occluded can be accurately located during life.

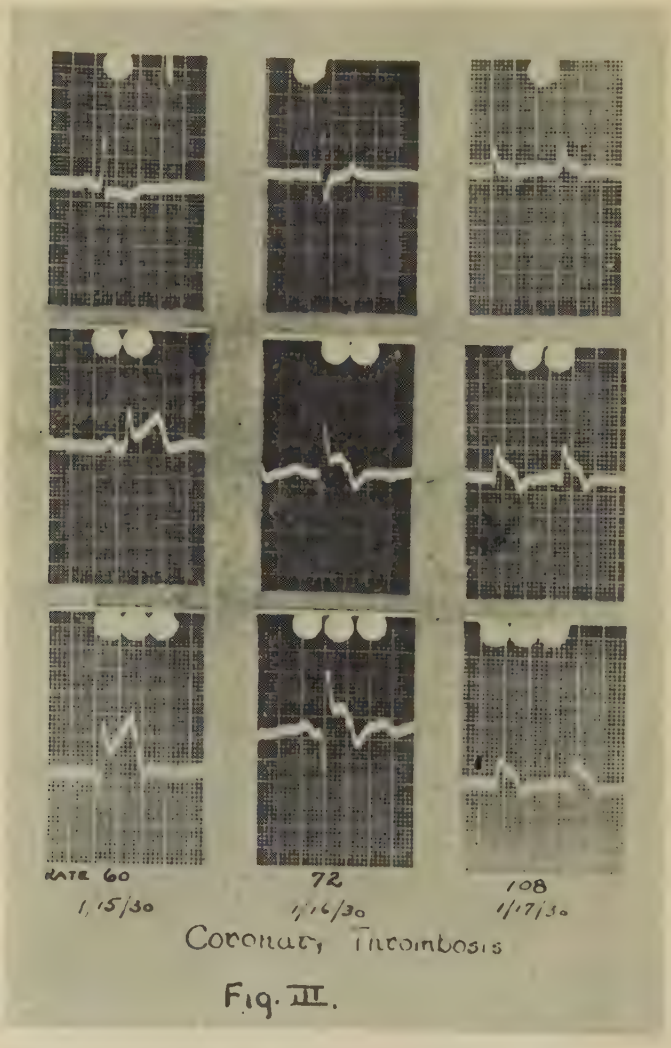


FIG. 3. Daily tracings from onset to death.

FIG. 4.

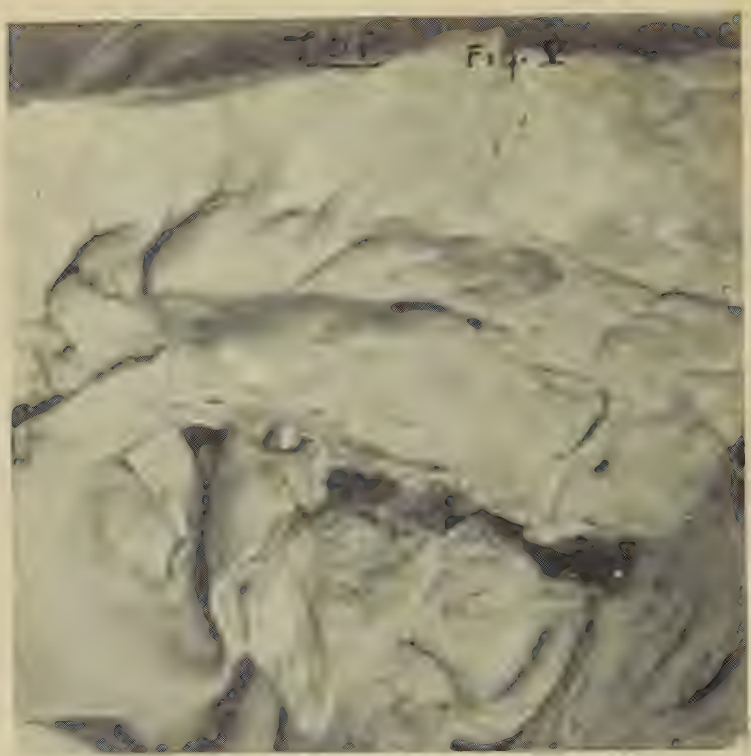


FIG. 5. Right coronary artery opened, showing the thrombus in the vessel.

FIG. 6. Posterior wall of the heart, showing thrombus extending as far as the posterior descending artery.

4915

Seasonal Variation in Hemoglobin.

VIRGINIA E. PLATT AND R. G. FREEMAN, JR.
(Introduced by E. G. Miller, Jr.)

From the Nursery School of the Child Development Institute, Teachers College, Columbia University.

The following study was undertaken to determine the possible seasonal variation of hemoglobin in a group of children between the ages of 21 and 44 months. The children in this study were attending a nursery school in New York. They came from a superior social group and their dietary and environmental conditions were above the average.

The method used for the hemoglobin determinations was that of Cohen and Smith.[1] The accuracy of this method had been previously checked by Karshan and Freeman.[2] The blood was taken in the morning between the hours of 10 and 11, and at the same period in each month.

TABLE I.

	Age	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May
1—L. B.	29 mo.	13.1	12.4	12.5	11.6	.....	11.3	12.1
2—R. C.	21 "	13.2	11.0	11.6	11.2	14.4	11.4	12.8
3—R. C.	21 "	13.1	12.0	12.1	10.8	13.3	13.3	12.5
4—M. E. B.	33 "	.....	13.3	12.1	.....	12.2	13.5	13.1
5—R. E.	29 "	14.2	11.4	11.4	11.0	11.6	11.4	14.7
6—C. F.	31 "	11.7	10.5	12.3	11.5	12.1	11.5	12.3
7—C. H.	26 "	15.0	10.5	10.0	11.7	12.6	10.8	12.9
8—M. L. H.	27 "	16.1	12.4	12.9	12.9	13.2	13.9	14.6
9—G. M.	34 "	14.1	11.8	11.2	11.2	12.3	12.7	12.8
10—G. P.	28 "	.....	11.3	11.0	11.5	13.2	11.6	11.8
11—J. R.	34 "	13.0	11.0	11.2	11.3	13.5	12.5	12.5
12—F. S.	25 "	14.3	12.4	12.2	11.4	13.4	12.9	13.0
13—D. T.	31 "	12.7	.....	11.7	.....	.....	10.9	12.6
14—H. A.	37 "	16.4	13.4	13.8	.....	.....	15.5	13.8
15—W. B.	35 "	.....	12.2	.....	12.2	.....	13.1	.....
16—A. D.	38 "	16.4	13.1	12.3	.....	.....	14.0	12.2
17—M. E. H.	38 "	13.6	11.6	11.2	10.2	12.8	10.9	12.8
18—J. H.	37 "	16.4	12.2	11.2	11.8	.....	13.3	11.0
19—J. H.	41 "	13.7	10.8	10.8	11.8	.....	13.3	11.4
20—J. H.	33 "	13.0	12.5	12.7	12.3	12.2	12.1	10.7
21—G. L.	39 "	16.6	12.0	12.1	12.4	.....	13.0	11.6
22—E. M.	35 "	13.0	12.4	12.4	11.0	12.9	.....	12.2
23—M. A. R.	44 "	15.3	12.0	13.8	.....	.....	14.1	11.8
24—D. S.	38 "	14.2	12.5	14.5	.....	.....	.....	13.7
25—D. S.	33 "	13.5	11.4	11.2	9.8	.....	12.4	12.8
26—N. S.	44 "	16.6	12.1	12.5	11.9	.....	.....	11.4
27—W. W.	35 "	15.0	12.2	11.9	12.5	12.8	12.9	11.1
28—M. W.	39 "	15.0	11.1	11.7	.....	.....	.....	12.4
29—G. W.	33 "	16.4	11.6	11.3	12.6	13.9	14.3	12.5

[1] Cohen, B., and Smith, A. H., *J. Biol. Chem.*, 1919, xxxix, 489.

[2] Karshan, M., and Freeman, R. G., Jr., *J. Lab. and Clin. Med.*, 1929, xv, 74.

Table I shows the individual figures (gm. of hemoglobin in 100 cc. of blood) for each child for each month that he was under observation. The age given is that at the time the first determination was made.

Williamson[3] using the spectrophotometric method showed that the sex difference at this age was not significant, and in obtaining the averages shown in Table II the 2 sexes were used together. The same author also showed the small difference between the values in the second and third and fourth years, so that in this paper all ages were used together.

TABLE II.

	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May
Average	14.4	11.9	12.0	11.5	12.8	12.8	12.6

It will be seen that, without exception, the February figures are all below those of November and that there is a rise in May but not to the November point, suggesting that the peak is probably during the summer months. The low point corresponds to the time of greatest incidence of upper respiratory tract infections[4] and to that of slowest growth[4] suggesting some common factor at work, probably to be found in environmental conditions to which city children are subjected during the winter months.

4916

The Stimulating Efficiency of the Normal Primary Alcohols.*

WILLIAM H. COLE AND JAMES B. ALLISON.

From the Physiological Laboratory of Rutgers University, New Brunswick.

As reported recently,[1] the rhythmic movements of the cirri of barnacles are sensitive indicators of environmental stimulation. A study has been made of the stimulating efficiencies of the first 4 normal primary aliphatic alcohols on *Balanus tintinabulum.*† The

[3] Williamson, *Arch. Int. Med.*, 1916, xviii, 505.

[4] Emerson, H., *J. Am. Med. Assn.*, 1927, lxxxix, 1326.

* Part of the expenses of this study has been met by grants from the American Association for the Advancement of Science (1929), and from the Society of Sigma Xi (1929-30), for which grateful acknowledgement is made.

[1] Cole, W. H., *J. Gen. Physiol.*, 1929, xii, 599.

† The experiments on *Balanus* were done at the Hopkins Marine Station, Pacific Grove, Calif., to the director of which the senior author is indebted for many courtesies.

TABLE I.

Minimum stimulating concentrations of alcohols for *Balanus*—Temp. 18.0±0.5°C.

Alcohol	Threshold Concentration	Concentration calculated according to Traube's rule.
Methanol	0.06 M	(0.06)
Ethanol	0.017	0.02
Propanol	0.0067	0.0066
Butanol	0.0027	0.0022

threshold concentration of each alcohol was determined under constant conditions of illumination, temperature and rate of flow, and in the absence of mechanical stimulation. Under such conditions it may be assumed that the stimulating agent is furnishing the minimum amount of energy necessary to activate the receptor. The effects of any secondary processes initiated by excessive stimulation are thus avoided, and the interpretation of the stimulating process is less likely to be erroneous. From the data in Table I it is clear that the stimulating efficiencies of the alcohols increase about 3 fold as each CH_2 group is added, beginning with methanol. This result is similar to that found by previous investigators in studying narcosis, toxicity and related effects. A review of the work and the theories on narcosis has been recently presented by Traube.[2]

We have made similar studies on the frog, *Rana pipiens*. Protecting the animal from all external stimuli, except the alcohol being studied, it has been possible to determine equally stimulating concentrations of the first 5 members of the normal primary aliphatic alcohols. Constancy of reaction time was the criterion for judging equally stimulating solutions. The data, presented in Table II,

TABLE II.

Reaction times of frog stimulated by alcohols at concentrations which are not excessive. Temp. 18.0±0.5°C.

Alcohol	Concentration	Reaction Time (sec.)
Methanol	1.8 M	22.3
Ethanol	0.6	26.5
Propanol	0.2	25.9
Butanol	0.066	32.5
Pentanol	0.022	32.4

show that the stimulating efficiencies increase with the number of CH_2 groups. The agreement of these 2 sets of data with the so-called Traube's rule is striking, but we would like to point out that interpretations of stimulation based on surface tension effects alone are probably incomplete. A more satisfactory account is possible by

[2] Traube, J., *Arch. f. Physiol.*, 1927, ccxviii, 749.

applying Langmuir's[3] "principle of independent surface action," according to which the distribution and orientation of polar organic molecules at an interface are determined by the character and number of the more active and the less active portions of the molecules. Throughout the alcohol series the potential of the polar group remains practically constant; *i. e.,* the effect of the OH group is constant, so that the increasing stimulating effect is determined by the non-polar group. The non-polar groups in the alcohol series differ in the number of CH_2 units, or in the length of the carbon chain. It is assumed that each additional CH_2 unit exerts an exponentially increasing effect on the shift of molecular equilibria at the receptive surface. This disturbance of the previously existing equilibria is the initial process of a catenary series of events which culminates in the discharge of a nervous impulse. Details as to the nature of the first effect are as yet unknown.

From our point of view it should be possible to secure data on the stimulating efficiency of the members of various homologous series of compounds, and by a study of the data from the different series, to formulate a generalized statement concerning the nature of the initial process in chemical stimulation.

Studies are now in progress on the effects of normal primary aliphatic aldehyde and acid series. A complete report of the whole work will be presented later.

4917

Hemorrhagic Reactions in Tuberculous Lesions and Skin Tests During Protracted Anaphylactic Shock.

L. DIENES.

From the Von Ruck Research Laboratory for Tuberculosis, Asheville, N. C.

The method which gives the best result for reproducing the observations which we will describe is as follows: Guinea pigs are injected intraperitoneally with a large dose (10-20 mgm.) of the slightly virulent tubercle bacillus strain R.1, or with killed tubercle bacilli. The infection is followed 3 to 8 days later by the intraperitoneal injection of 0.1 to 1.0 mgm. egg white (dry weight). After this treatment the guinea pigs usually develop a strong skin sensitiveness to egg white,[1] often giving large necrotic skin reactions with 0.01 mgm. egg white.

[3] Langmuir, I., *Chem. Reviews,* 1929, vi, 451.

[1] Dienes, L., *J. Immunol.,* 1929, xvii, 531.

When the guinea pigs, 9 to 14 days after the treatment, are injected with somewhat larger doses of egg white intraperitoneally (10-30 mgm. dry weight) they die in 4-12 hours, the symptoms showing great similarity to the tuberculin shock. The most striking finding at the autopsy—beside an abundant exudate in the peritoneal cavity—is that in the peritoneum and the tuberculous lesions (in the omentum, mesenterium, and peritoneal walls) large hemorrhagic areas are present. Sometimes the hemorrhages extend almost over the entire peritoneum. When the reaction is slight it corresponds entirely to the usual reaction of tuberculous animals killed by the injection of tuberculin around the tuberculous lesions. From 93 tuberculous guinea pigs sensitized with egg white, tested with intraperitoneal injections during the last year, 30 died with the above described symptoms. In these guinea pigs the method of treatment and sensitization and also the interval between the treatment and the testing was variable. Of the guinea pigs which were infected and treated intraperitoneally, with few exceptions all died with the characteristic symptoms when tested in the third week after the treatment.

If the guinea pigs live somewhat longer or survive a severe shock we often observe that the formerly made skin tests with egg white and tuberculin show a bluish purple discoloration, and also extensive hemorrhages often occur in them. The hemorrhagic skin areas or a part of them might become necrotic during the following days. We observed extensive hemorrhages also in recently healed operation sites. In one guinea pig a strong reaction was present in skin areas where the hairs had been pulled out during the shock. In an experiment which might serve as an illustration 8 guinea pigs were injected intraperitoneally with egg white 23 days after sensitization. These guinea pigs were infected in the groins. All developed a severe protracted shock, 5 dying between 2 hrs. 20 minutes and 20 hrs. The guinea pigs were skin tested the day before in 4 different sites with tuberculin preparations. In 5 guinea pigs, 2 of which survived, smaller or larger hemorrhagic areas developed in the skin tests. Since our attention has been called by the paper of Shwartzman[2] to this phenomenon we observed it accidentally in 3 guinea pigs after tuberculin injection among a large number of tuberculin-tested guinea pigs. Two experiments in which we tried to reproduce this phenomenon with tuberculin in guinea pigs remained unsuccessful.

The hemorrhagic reaction in our observations was specific neither

[2] Shwartzman, G., PROC. SOC. EXP. BIOL. AND MED., 1928, XXV, 560; 1928, XXVI, 207.

to the tuberculous lesions nor to the inflammatory reaction (skin test) produced with egg white. The readiness to form hemorrhages in inflammatory areas of various origin is very probably a symptom of the severe illness caused in the sensitive animals by the egg white or tuberculin, which is present in varying degree in the different animals.

There are 2 reasons why we publish these observations before the completion of the work—concerning the general reactions of the tuberculous animals sensitized with egg white—in connection with which they were made. The first is that the observations in their present form make it very probable that the focal reactions which we observe around the tuberculous lesions in the animals killed in tuberculin shock are not the result of a special sensitiveness of the lesions to the tuberculin. There is no reason to doubt the specificity of the tuberculin reaction, but the focal reaction, at least in part, seems to be a non-specific symptom of a certain type of intoxication of the organism. It is well known that focal reactions in tuberculous patients are often caused by various non-specific influences. The other reason for the publication of these observations is that they are in close analogy with the intensification of skin reactions in rabbits after the intravenous injection of bacterial filtrates, as described by Shwartzman[2] and Hanger.[3] The doses of the bacterial filtrate which produced this effect are toxic, (a large percent of the rabbits die after the intravenous injection) and it is possible that the hemorrhagic reaction in the formerly injected skin areas is a result of the intoxication, as seems to be the case in our observations.

4918

Average Valence of the Gelatin Ion Determined by a Modified Theory of Membrane Equilibrium.

R. BEUTNER.

From the Department of Physiology and Pharmacology, University of Louisville School of Medicine.

From Donnan's theory[1] we can calculate the depression of the osmotic pressure of a gelatin solution, by the addition of a salt like NaCl. According to this theory this depression should never exceed 50% of the pressure of the colloid electrolyte in complete absence of a diffusible electrolyte. Experimental observations show

[3] Hanger, F. M., PROC. SOC. EXP. BIOL. AND MED., 1928, xxv, 775.

[1] Donnan, F. G., *Z. f. Elektrochem.*, 1911, xvii, 572.

that this is far from being true. As shown by Loeb[2] and others, salt addition depresses the osmotic pressure of gelatin chloride frequently to less than one-tenth of its original size.

Manifestly such a deviation must be due to an insufficiency of the premises of the theory, probably to the too simple assumption of Donnan that the colloidal micellae carry single electrical charges. Calculations based on the assumption of the presence of multiple charges of the colloidal ion—to be published *in extenso* later—show that the *fraction of osmotic pressure which remains after the maximal lowering by a salt excess amounts to* $1/n+1$ *of the pressure in absence of a diffusible electrolyte—where* n *signifies the valence of the colloidal ion;* hence the pressure is depressed to one-half for monovalent, 1/3 for bivalent, 1/11 for 10 valent colloidal ions, etc.

From Loeb's measurements[2] we can figure the osmotic pressure of gelatin chloride by adding to the observed values of osmotic pressure the counterpressure which is due to unequal distribution of the $H^+=$ ions on either side of the membrane. This latter magnitude can also be figured from Loeb's data. By the addition of an excess of HCl practically the entire gelatin is transformed into gelatin chloride. For a 1% solution of gelatin chloride the osmotic pressure is thus figured as about 1520 mm. water column. By the addition of an excess of NaCl this is depressed to as low a value as nearly 23 mm.—all these data being taken from Loeb. The pressure is depressed, therefore, to about 1/66, hence, the gelatin ion must carry approximately a charge 67 times larger than a H^+ or $Cl^- =$ ion. This can only be meant as the average charge since colloidal micellae are never of uniform size; gelatin, moreover, is chemically inhomogeneous as proven by Kunitz and Northrop,[3] hence probably of varying composition, and of widely varying molecular size.

4919

The Action of Soaps in the Animal Body.

IRVINE H. PAGE AND EDGAR V. ALLEN.

Aus der chemische Abteilung der Deutsche Forschungs-anstalt für Psychiatre (Kaiser Wilhelm Institute) München.

In the animal organism the possibility of the formation of soaps from fatty acids and various bases is often present. It is believed

[2] Loeb, J., "Proteins and Colloidal Behavior," New York, 1922.

[3] Kunitz, M., and Northrop, J. H., *J. Gen. Physiol.*, 1929, xii, 379.

by many that soaps formed in the intestine are absorbed as such and act as a means of fat transport in the blood. In many types of pathological process, soap may be produced, as for instance in atherosclerosis, acute hemorrhagic pancreatitis, necrosis of the brain, etc. Soap is often used as an abortifacient, in various therapeutic measures, in the preparation of vaccines, and selective bacterial cultivation.

We have, therefore, made a study in mice, rats and rabbits of the activities of various of these substances. The following soaps were studied: The sodium, monoethanolamine, diethanolamine and triethanolamine salts of capric, lauric, oleic, linoleic, and ricinoleic acids (n/20 aqueous solutions at pH 7.4). This is a preliminary paper, the detailed work appearing in the *Naunyn-Schmiedeberg Archive für experimentelle Pharmakologie.*

Studies on 500 mice, intravenously injected, show the following order of toxicity, the amount required to produce death in 24 hours being taken as the M.L.D. Mono, di and triethanolamine ricinoleate > Na ricinoleate and Na laureate > Na oleate, Na linoleate, mono and diethanolamine oleate, and monoethanolamine caprinate > triethanolamine oleate > Na linoleate and diethanolamine caprinate > triethanolamine caprinate and Na butyrate. The amounts required to produce death within 24 hours when the soaps were injected intraperitoneally were from 2-10 times as great.

Pathologic studies of mice dying within 24 hours as the result of a single soap injection have shown so far only marked and uniform congestion of the viscera and in many cases hemorrhage into the pulmonary alveoli. Large doses of soap are required to produce very evident hemolysis.

Studies on 60 rats injected intraperitoneally 3 times weekly for 6 weeks (50 mg. soap per kg.) showed marked variation in the response to different soaps. Peritoneal adhesions, fatty and degenerative changes in the liver, and degenerative changes in the kidneys were often present. There appears to be a moderately specific response to the individual soaps. It is interesting to note that whereas it is usually assumed that soaps are relatively harmless physiological substances, they are toxic when parenterally injected and the introduction of these substances is followed by a train of pathological tissue changes.

Blood pressure studies with 12 rabbits under amytal anesthesia clearly demonstrate very marked and often prolonged fall in pressure depending on the soap used. Some of the soaps produced very evident rhythm changes especially the ricinoleate soaps. Most of the soaps act to stimulate respiration to a marked degree.

The effect of diethanolamine ricinoleate (2 cc. n/20 solution intravenously) on the permeability of the meninges was tested in 12 experiments with rabbits, using Uranin A as circulating dye. Occipital puncture was performed 3 hours after the intravenous injection of soap and dye. In every case there was a small but definite increase in the dye which was found in the cistern fluid as compared with the fluid from animals in which the dye alone was injected.

4920

Dissociation of Allergy from Immunity in Pneumococcus Infection.

ARNOLD RICE RICH AND J. HOWARD BROWN.

From the Department of Pathology and Bacteriology, The Johns Hopkins University.

It is commonly believed that allergic hypersensitiveness plays an important and necessary rôle in acquired immunity to bacteria. The idea behind this belief is that the rapid allergic inflammation, which occurs at the site at which bacteria lodge in the sensitized body, prevents the spread and causes the death of the bacteria, while in the non-allergic body in which inflammation develops with less speed and intensity, the bacteria are able to proliferate and to spread more rapidly over the body, being less hampered by the antagonistic forces inherent in the process of acute inflammation. It has, however, been pointed out[1] that there are numerous reasons for questioning the assumed necessity of allergy for the operation of immunity; and, further, that in certain infections, if not in all, it is of considerable importance to know whether or not allergic hypersensitiveness is really necessary for immunity, since tissue destruction is so frequently a direct result of hypersensitiveness of the cells to bacterial products which are relatively innocuous to unsensitized tissues. Rich, Chesney and Turner[2] have produced immunity in the absence of allergy in experimental syphilis. The present study demonstrates that allergy is not necessary for immunity in pneumococcus infection.

A high degree of allergy in rabbits actively immunized to the pneumococcus can easily be demonstrated by intracutaneous tests. In the present attempt to dissociate allergy from immunity, however,

[1] Rich, *Arch. Int. Med.*, 1929, xliii, 691.

[2] Rich, Chesney and Turner, to be published.

we have made use of the method of passive immunization. The test rabbits were given intravenously an immune serum prepared against a Type I pneumococcus, and control rabbits were given the same amount of normal serum. Both test and control animals were then inoculated intracutaneously with various multiples of the lethal dose of a Type I pneumococcus of tested virulence for rabbits. The sites of inoculation were carefully studied at frequent intervals, and in no instance did an allergic reaction occur at the site in the immunized animals, whereas progressive, destructive lesions developed in the controls. The latter died, with septicemia, usually within 36 hours after the inoculation; exceptional animals, however, lived as long as 72 hours. On the contrary, all of the immunized animals survived without developing any appreciable lesion at the site of inoculation. Twenty-two passively immunized animals and the same number of controls have been used in these experiments.

It is clear, therefore, that there exists in the plasma of an animal immunized against the Type I pneumococcus, a means of protection against this organism which is effective in the absence of allergic inflammation. It is also clear that the widely postulated necessity of a local allergic sacrifice of tissue for the preservation of the body as a whole during the operation of acquired immunity finds no support in these experiments.

Conclusions. Following the intravenous injection of an immune serum prepared against the Type I pneumococcus, immunity to infection can be demonstrated in the absence of allergic inflammation. The inflammation of allergy, therefore, is not necessary for the operation of immunity in this infection.

4921

A Study of the Islands of Langerhans in vivo With Observations on the Circulation.

BENJAMIN N. BERG.

From the Department of Pathology, College of Physicians and Surgeons, Columbia University, New York City.

Studies of the pancreas with an intact circulation were made in the living animal by Kuhne and Lea,[1] Mathews[2] and Covell.[3]

[1] Kuhne, W., and Lea, A., *Untersuch. a. d. Physiol. Instit. d. Univ. Heidelberg*, 1882, ii, 448.

[2] Mathews, A., *J. Morphol.*, Supplement, 1899, xv, 171.

[3] Covell, W. P., *Anat. Rec.*, 1928, xl, 213.

The following report deals with the physiology of the islands of Langerhans in the pancreas of a living mammal with observations on the circulation. The pancreas of the white mouse was the most satisfactory for this purpose. Male mice weighing from 10 to 20 gm. were used. Anesthesia was induced by the subcutaneous injection of 1 mgm. of sodium amytal (Eli Lilly & Co.) per 5 gm. of body weight. After 10 to 20 minutes narcosis was complete. The animal was placed upon its right side in a petri dish that rested upon a warm stage. An incision measuring 1 cm. was made in the left flank, and the anterior pole of the spleen was grasped and drawn through the opening in the abdominal wall. By gentle traction and rotation the tail of the pancreas was brought into view, attached to the hilum of the spleen. After teasing the lobules apart to expose the main central vessels, the pancreas was bathed with physiological saline at 37°C. and covered with a small cover slip. Observations extended one to 2 hours under a binocular microscope using direct illumination.

In suitable preparations the islands could be identified by the naked eye, as minute white dots on a yellow background. They were distributed usually along the course of the main central vessels or branches of the latter. Microscopically they appeared as brilliant yellowish white bodies on the surface of the less refractile yellow pancreas or on a vein. They were round, oval or kidney-shaped, had well defined margins and varied in size from 0.6 to 0.07 mm. No spontaneous changes in the number, size or shape of the islands were noted. Islands that were studied *in vivo* were identified by serial sections.

Accurate observations were possible only when the islands were situated at the surface of the pancreas, although when they were imbedded deeper in the gland, they could be distinguished by the fact that they reflected the light more brilliantly than did the surrounding tissue. Great anatomical variations existed in different animals with respect to the number of islands that were superficial. In the glands of some mice, many islands were found at the surface. In others only one or two were available for study. In some pancreas no islands were found at the surface.

The circulation in the islands was distinguished clearly. The capillaries appeared as thin, red, thread-like loops that had U. S. V. or spiral shapes and dipped into the substance of the islands. The capillaries were situated between double columns of highly refractile islet cells. The number of visible capillaries varied in different glands. Sometimes no vessels were seen. As a rule the main capil-

lary pattern remained unaltered although lightning-like changes were observed frequently, now one, now another capillary becoming visible and then disappearing from view. The circulation was extremely rapid and the red blood cells were not distinguished. The direction of the flow of blood was often noted in the efferent venules at the periphery of the islands. In this region changes in the course of the flow of blood through different capillaries occurred at irregular intervals. Anastomoses between the capillaries of the island and the interacinar capillaries were seen. In a few instances the afferent arterioles were distinguished, but as a rule they were not visible. Rhythmic spontaneous contractions were observed in the small arteries. In studying the circulation it was important to determine whether any pancreatic tissue was interposed between the island and the surface, because the interacinar capillaries were easily confused with those in the underlying islands. The anatomical arrangement of the circulatory system in the islands of Langerhans was investigated by Dr. Jas. P. Beck by means of the double injection dye method. It was found that one or more afferent arterioles and one or more efferent venules were connected with each island; in addition anastomoses existed between the capillaries in the islands and those in the adjacent acinar tissue. These findings corresponded with the observations which had been made *in vivo*.

Observations were also made concerning the action of epinephrin (Armour & Co.) and pituitrin (Armour & Co.) on the blood vessels of the pancreas, including the circulation in the islands of Langerhans. The drugs were injected intravenously into the left lateral tail vein in doses varying from 0.01 cc. to 0.05 cc. Vasoconstrictor effects on the arteries were noted with epinephrin in dilutions of 1:10,000, 1:20,000, 1:100,000, 1:500,000 and 1:1,000,000. Following the injection of epinephrin in the higher orders of concentrations, blanching and cessation of the circulation in the islands were observed. With the more dilute solutions the effects upon the circulation in the islands were irregular and difficult to follow. This was due to the fact that the vasoconstrictor influence of epinephrin in high dilutions was very transitory.

Vasoconstrictor effects upon the arteries similar to those following epinephrin were seen after the injection of pituitrin in dilutions of 1:400, 1:1000, 1:10,000, 1:100,000 and 1:200,000. These were associated with changes in the circulation in the islands. With doses in the order of 0.02 cc. of 1:10,000 and 0.03 cc. of 1:100,000 dilutions, irregular and segmental vasoconstrictor reactions of the small arteries were noted at the branching points of the vessels. Pituitrin

was tolerated by the mice in much higher concentrations than epinephrin.

During vasoconstrictor action the capillaries in the islands were distinguished as thin, grey channels lying between interlacing cords of cells; the islands became lusterless and their outlines grew indistinct.

The results of the studies with epinephrin and pituitrin indicate that the circulation in the islands of Langerhans is probably regulated by changes in the afferent arterioles or the small arteries in response to physiological stimuli. This may constitute the mechanism by which the supply of insulin to the circulation is controlled. In diabetes the amount of insulin available to the body may be curtailed by vasomotor disturbances in the arterioles and small arteries without demonstrable lesions in the islands.

4922

Cytological Changes in the Definitive Ova of the White Rat.

THEODORE J. MILLER. (Introduced by T. C. Nelson.)

From the Histological Laboratory, Rutgers University.

It has been noted in atretic follicles of ovaries of a number of different mammals that the oocytes appear to be going through a process closely resembling normal development. It appears to be a matter of interpretation, after a review of the literature from 1884-1930, as to whether this process is the beginning of parthenogenetic cleavage or whether it is only degenerative fragmentation.

Segmented ovarian ova appeared in every stage of the oestrous cycle of the white rat. They were more numerous in pregnant rats which were at the fifth and eighteenth day of their gestation period. This observation agrees with that of Evans and Swezy.[1] The small size of the follicle and of the segmented egg within, in comparison with the normal follicle and egg is striking. The abnormal follicles were in every case below the surface of the ovary, and in nearly every case they were adjacent to larger normal follicles, or to ovarian corpora. Swezy[2] found very few follicles developing from the germinal epithelium bounding follicles or corpora, due she believed, to the

[1] Swezy, O., and Evans, H., *Science*, 1929, cxxi, 46.

[2] Swezy, O., *J. Morph.*, 1929, xlviii, 445.

tension exerted by the expanding body beneath. As the normal follicle enlarges it pushes out in lobules and due to these outpushings on the part of the normal follicle normal growth and development is denied the smaller adjacent follicles. As pressure is increased upon a follicle there is a corresponding increase in pressure upon the ovum within. It seems probable that this increase in pressure has some effect upon the initiation of the development of the egg.

In 39 of the 42 cases studied the segmented ova had a constant diameter of either 0.0428 mm. or 0.0570 mm. Kirkham and Burr[3] gave the diameter of the living unsegmented egg as of 0.079 mm. It is evident from this that when the egg is prevented by the surrounding pressure from growing larger than 0.0570 mm., development is started.

The egg is located deep within the ovary and in most cases is prevented from reaching the surface by an intermediary normal follicle, which in turn as it grows, exerts a pressure upon its neighbor. As a result of this increase in pressure it appears that the egg follows the path of least resistance and starts to divide. It would logically follow that if the pressure on the egg increases, the "blastomeres" would eventually separate. This is exactly the result as seen in the number of eggs studied. If, however, this pressure upon the egg is not increased by the surrounding "agent," the tendency for the egg would be to continue dividing without a complete separation of the constituent blastomeres in the mother liquor of the follicle.

The question arises as to whether the egg divides in normal cleavage stages or whether it is degenerative fragmentation. One would naturally expect in the circumscribed area of an ovarian follicle that the embryonic development would be very defective, especially in the presence of an abnormal pressure upon the egg. In the 60 eggs studied which showed segmentation, division seemed to be more or less regular. The 2, 3, 4, and 8-cell stages indicate a decided tendency towards normal development. What occurs after the 8-cell stage is uncertain, but from my own material I believe that degenerative fragmentation is at work. No traces of further embryonic development as found in the guinea pig by Loeb[4] could be found in any of my material.

As a result of this study of some 60 segmented ovarian ova and of the follicle surrounding each, it is shown that normal growth and development were denied them due to the presence of intermediary

[3] Kirkham, W. B., and Burr, H. S., *Am. J. Anat.*, 1913, xv, 291.

[4] Loeb, L., PROC. SOC. EXP. BIOL. AND MED., 1930, xxvii, 413.

follicles, atretic follicles or of corpora lutea. Further, this condition causes an increase in pressure upon the follicle, as a result of which there is a corresponding increase in pressure upon the egg. Since this condition has been found in each of the 60 eggs studied, it is concluded that this increase in pressure plays some part in the initiation of development of the ovarian ova as found in the white rat.

4923

Factors Determining the Ergosterol Content of Fungi.

PAUL S. PRICKETT, OLIVER N. MASSENGALE, WARREN M. COX, JR., AND CHARLES E. BILLS.

From the Research Laboratory, Mead Johnson and Company, Evansville, Indiana.

Since 1927 this laboratory has been engaged in the development of high-yielding ergosterol sources. The fungi being characterized by a relatively large ergosterol content, numerous representative species were investigated and the factors influencing the elaboration of ergosterol determined.

We used 25 true yeasts, 4 pseudo yeasts, 18 molds, 3 mushrooms, and 2 bacteria. We found that the inherent ergosterol producing capacities of the different species vary enormously, and that by manipulating the cultural conditions these capacities may be attained or repressed.

The bacteria, bovine and human types of *Mycobacterium tuberculosis,* showed no ergosterol by spectrographic assay (*cf.* Prickett, Massengale, and Cox[1]). Also Anderson and Chargaff[2] found no cholesterol or any substance giving sterol color reactions in the unsaponifiable matter from a human type of *M. tuberculosis.* Apparently this is the first one of the fungi in which no ergosterol has been found; the absence of ergosterol is all the more surprising when the high lipoid content of this organism is considered. Of the other fungi the yeasts showed the widest variation. *Saccharomyces logos* contained but a trace, while *S. carlsbergensis* developed 2.4% of ergosterol. Spectrographic analyses and actual extractions of the more promising molds—representatives of the *Mucors, Penicillia,* and *Aspergilli*—and also the pseudo yeasts and mushrooms, gave values between the extremes of the yeasts. It is interesting to note

[1] Prickett, P. S., Massengale, O. N., and Cox, W. M., Jr., *J. Bact.*, 1930, xix, 8.

[2] Anderson, R. J., and Chargaff, E., *J. Biol. Chem.*, 1929, lxxxiv, 703.

that Heiduschka and Lindner[3] found in a *Penicillium* 0.8% of ergosterol colorimetrically, which is the same value that we obtained spectrographically with a *Penicillium* cultivated in one of our media.

In general, we found that for a given fungus a neutral or slightly alkaline medium was conducive to vigorous growth and relatively large ergosterol production. An abundant air supply was not only favorable but essential. Temperature *per se* did not seem to be an important factor, but in connection with time and the available supply of nutrients it exerted an influence. We have so far been unable to observe that salts other than those essential for the vigorous growth of the organisms exert any marked effect; however, we found that the concentrations and combinations of salts had noticeable effects, especially on the amount of growth obtained. A good source of carbohydrate and nitrogen was found in beet molasses, or a mixture of beet molasses with other sugars, or such mixtures with added urea. In most cases high ergosterol percentage was associated with vigorous growth, but not all cultures which grew vigorously elaborated large amounts of ergosterol.

4924

Effect of Oestrin on Gonad Stimulating Power of the Hypophysis.*

R. K. MEYER, S. L. LEONARD, FREDERICK L. HISAW AND S. J. MARTIN.

From the Zoology Department, University of Wisconsin.

Smith and Engle[1] showed that the anterior lobe of the hypophysis of the guinea pig in oestrum is less potent in its gonad stimulating power than the hypophysis of animals in the dioestrum. Burch and Cunningham[2] reported that injection of a commercial placental extract, containing considerable amounts of oestrin, into adult, castrate, female rats tends to increase the gonad stimulating power of the pituitaries of such animals, as compared with non-injected castrate controls of approximately the same weight. The period of injection in their experiment was 6 days and the dosage employed was from

[3] Heiduschka, A., and Lindner, H., *Z. Physiol. Chem.*, 1929, clxxxi, 15.

* Assisted by grants from the National Research Council, Committee on Problems of Sex.

[1] Smith, P. E., and Engle, E. T., *Anat. Rec.*, 1929, xlii, 38.

[2] Burch, J. C., and Cunningham, R. S., PROC. SOC. EXP. BIOL. AND MED., 1930, xxvii, 331.

5-25 R.U. during the injection period. The potency of the pituitaries was determined by implanting them into immature female mice. This report appeared while we were conducting experiments to ascertain the influence of long periods of administration of the oestrous producing hormone on the ovaries and pituitaries of non-castrate immature rats, and the pituitaries of castrate adult male and female rats. This paper presents the data obtained in these experiments.

Thirty-four immature female rats varying in age from 30 to 40 days were injected with 2 R.U., 0.1 cc., per day of an oil soluble oestrous hormone prepared from the amniotic liquor of the cow.† Twenty-eight litter-mate sisters of these experimental animals were injected with 0.1 cc. of Mazola oil and used as controls. The period of injection varied from 30 to 70 days, after which the animals were sacrificed, ovaries weighed and pituitaries implanted into female rats varying in age from 20 to 30 days. Two pituitaries were implanted simultaneously into each recipient. The criterion of gonad stimulating power of the hypophyseal implants was the time necessary for the opening of the vaginas of the recipients. This method is not satisfactory unless the vaginas of the recipients being compared open 4 or more days apart. The ovaries of the oestrin injected donors weighed 40% less than the ovaries of the control litter-mate donors. The pituitaries of the oestrin injected rats opened the vaginas of the recipients from 9 to 25 days after implantation, whereas the vaginas of control recipients which received pituitaries from the control donors opened 4 to 5 days after implantation.

Evans and Simpson[3] have found that the gonad stimulating power of the pituitaries of castrate males and females is much greater than those of non-castrated males and females. These data led us to study the influence of the oestrous producing hormone on the hypophysis of castrate animals. The gonads were removed from 15 adult males and 19 adult females of approximately the same weight. Of these castrate animals 7 males and 8 females were used as controls. These control animals were not injected with Mazola oil, as we found that it did not influence the gonad stimulating power of the hypophysis. The remaining castrate males and females were injected with 4 R.U., 0.1 cc. of oil soluble oestrin per day for 31 days after which their pituitary glands were implanted into immature litter-mate female rats between the ages of 20 and 30 days. One donor pituitary was implanted into each of the recipients. In this experiment the vaginas of the control, and experimental recipients

† The oestrous hormone was kindly furnished by E. R. Squibb & Sons.

[3] Evans, H. M., and Simpson, M. S., *Am. J. Phys.*, 1929, lxxxix, 371.

opened at about the same time and the ovaries were weighed one day later. A comparison of the weight of the ovaries from animals which received implants from control and experimental male donors demonstrated that the ovaries of the experimental recipients weighed 28% less than the ovaries of the controls. The ovaries of the experimental recipients which received pituitaries from oestrin injected castrate females weighed 35% less than the ovaries of the control recipients.

The data from the experiments on the immature rats demonstrate that the oestrous producing hormone inhibits the normal development of the ovary and decreases the gonad stimulating power of the hypophysis. This suggests that the influence of oestrin on the ovaries of immature animals is due to its action on the hypophysis.

The results obtained in the experiments on the adult castrate males and females, demonstrate that oestrin as administered decreases the gonad stimulating power of the hypophysis and that the hypophysis of the female seems to be more susceptible to the oestrous producing hormone than that of the male. Whether the oestrin inhibits the production or storage of the gonad stimulating substance of the anterior lobe of the hypophysis is now being investigated.

4925

The Ciliary Systems in the Oviduct of the Pigeon.*

G. H. PARKER.

From the Zoological Laboratory, Harvard University.

Over a year ago I described the ciliary systems in the oviducts of the painted turtle.[1, 2] These ducts are lined from the infundibulum to the distal outlet with ciliated epithelium. In the proximal portion of the ducts the cilia form two systems, a general one, the abovarian, which sweeps from the ovary toward the exterior and which covers most of the inner surface of the duct, and a restricted one, the proovarian, a narrow longitudinal band not more than 2 mm. wide, in which the cilia beat toward the ovary. As was pointed out in these earlier publications, the spermatozoa can make no headway against

* This research was carried out with funds received in part from the National Research Council, Committee for Research in Problems of Sex.

1 Parker, G. H., PROC. SOC. EXP. BIOL. AND MED., 1928, xxvi, 52.

2 Parker, G. H., *Am. J. Physiol.*, 1928, lxxxvii, 93.

either of these systems but are swept up or down the duct in accordance with the system with which they are associated. It is clear from the action of these cilia that the pro-ovarian system must be concerned with the transportation of the sperm cells to the ovary and the abovarian with the clearing of the oviducts and possibly with the transportation of the eggs toward the exterior. It is a matter of interest to ascertain to what extent these two systems are represented in the birds and in the mammals.

The oviduct of the pigeon was studied with this question in mind. It is comparatively easy in this oviduct to distinguish the 5 conventional parts, namely, the infundibulum next the ovary, the extensive albumen-producing portion of the duct, the somewhat restricted isthmus, the so-called uterus or shell-producing part, and, at the distal end, the vagina. All these parts in the pigeon as in the turtle are covered with a ciliated epithelium whose cilia for the most part beat from the interior toward the exterior. They, therefore, reproduce what has been called in the turtle, the abovarian system. Over the albumen-producing portion of the duct a second system can easily be detected. This system is a band of approximately one-fourth the width of the duct in which the cilia persistently beat toward the ovary. It, therefore, reproduces the pro-ovarian system of the reptile. It is best demonstrated by exposing a transverse section of the appropriate portion of the oviduct by longitudinal splitting and by inspecting under the microscope the edge of the tissue when folded upon itself parallel to the length of the duct. By rolling such a preparation upon itself one may pass with ease from regions in which the cilia are seen to beat toward the exterior to those in which they beat toward the ovary. Thus the albumen-producing portion of the oviduct in the pigeon reproduces with great completeness the conditions found in the turtle.

If portions of the albumen-producing section of the pigeon's duct are exposed so that the complete transverse extent of the duct is open to view and the preparation is flooded with Ringer's solution and carmine, a close inspection under the microscope shows a pro-ovarian current on part of the wall and an abovarian current over most of it.

Spermatozoa placed in contact with either of these ciliary systems are swept in appropriate directions up or down the tube irrespective of the proper locomotion of the spermatozoa themselves. It is, therefore, plain that in the pigeon, as in the turtle, the spermatozoa must be carried from approximately the middle of the tube toward the ovary by the pro-ovarian system. How the sperm cells

reach this system from the lower part of the tube where they are deposited by the male is not wholly clear. There is much evidence to show that the oviduct of the bird exhibits more or less antiperistalsis. This activity has been called upon as an explanation of the formation of certain types of double eggs in that an egg fully formed and near the exit of the oviduct is by means of antiperistalsis returned to the upper part of the tube to be incorporated in a second egg in process of formation. The fact that feathers and other barnyard refuse may be occasionally included in eggs, as pointed out by Landois,[3] is to be explained by antiperistalsis. Probably antiperistalsis, associated with the locomotor activities of the spermatozoa themselves, accounts for the transfer of these cells from the position of their deposit at the distal end of the duct to the beginning of the pro-ovarian ciliary system by which they would then be carried to the neighborhood of the ovary.

In conclusion, it may be stated that the pigeon possesses in its oviduct in addition to the abovarian system of cilia a pro-ovarian system like that of the turtle except that it is somewhat more restricted.

4926

Effect of the Luxus Consumption of Meat Upon the Kidney of the Albino Rat.

ROBERT A. MOORE AND F. A. HITCHCOCK.

From the Departments of Pathology and Physiology, Ohio State University.

During the course of investigations on the effect of high protein (meat) diet on growth,[1] voluntary activity,[2] and nitrogen excretion during a fast,[3] some 43 animals became available for pathological examination. Of these animals 23 belonged to the high protein group, while the remaining 20 were controls. The two groups were of approximately the same age, but as a result of the difference in diet the high protein animals were about 20% heavier than the controls. The control animals were fed on our regular stock diet which consists of whole wheat flour, 62.5%, dry casein, 15%, skim milk powder (analac), 15%,

[3] Landois, H., *Fremde Einschlüsse in Huhnereiern.* 1882. Humboldt, i, 22.

[1] Hitchcock, F. A., *Am. J. Physiol.*, 1926, lxxix, 218.

[2] Hitchcock, F. A., *Am. J. Physiol.*, 1926, lxxix, 206.

[3] Hitchcock, F. A., and Rawlins, A. L., *Am. J. Physiol.*, 1927, lxxx, 450.

$CaCO_3$, 1.5%, cotton seed oil 5% and water, 10%. To this mixture an appropriate amount of cod liver oil was added. We have computed that this ration contains 29% protein, 57% carbohydrate, and 14% fat. Each animal in the experimental group in addition to this stock ration was given daily, 8 gm. of fresh lean beef cooked rare. The average food intake of the control group was 14.4 gm. daily, which equaled 55 calories, 29% of which was furnished by protein. Each experimental animal in addition to its 8 gm. of meat, ate 12.4 gm. of the stock ration daily. This made a total daily caloric intake of 64 calories, 36% of which was furnished by protein. The average daily urinary nitrogen for the control group was 0.78 mgm. while for the experimental group it was 1.51 mgm. per gram of body weight.

Dr. R. R. Durant determined, by the direct method, the blood pressure of 8 of the control and 7 of the experimental animals. In the control group systolic pressure varied from 102 to 130, with an average of 120 mm. of mercury. With the experimental animals the variation was from 103 to 129, with an average of 115 mm. of mercury. These figures do not vary significantly from the normal values for the blood pressure of the white rat as reported by Durant.[4]

Kidney weights were determined on 8 of the control and all of the experimental animals. These weights expressed in percentage of total body weight varied for the controls from 0.62 to 0.70% with an arithmetical mean of 0.64%. For the experimental group the variation was from 0.67 to 0.90% with an arithmetical mean of 0.73%. The overlapping was minimal. Only one control animal was equal to, or greater than, the lowest experimental animal while only 5 experimental animals were equal to, or lower than, the highest control. The average weight of the kidneys of the experimental group was 14% higher than that of the controls. This is 2.2 times greater than the probable error and the difference is therefore significant.

Histological examination was made of both kidneys of all animals. All of the animals in the control group showed normal kidneys. Of the experimental animals 17, or 74%, showed some pathological change. These changes consisted of focal areas of fibrosis and cellular infiltration in the cortex, dilatation of the cortical tubules with hyaline casts, and slight to moderate thickening of Bowman's capsule. None of these changes is analogous to human chronic diffuse glomerulonephritis.

We conclude, therefore, that in the albino rat a high protein

[4] Durant, R. R., *Am. J. Physiol.*, 1927, lxxxi, 679.

(meat) diet produces (1) no change in systolic blood pressure; (2) a hypertrophy of the renal substance; and (3) certain histological changes of a pathological nature which are not, however, analogous to chronic nephritis in man.

4927

Serum Calcium and Phosphorus of Guinea Pigs after Administration of Single and Repeated Doses of Parathormone.

AARON BODANSKY, JOHN E. BLAIR AND HENRY L. JAFFE.

From the Laboratory Division, Hospital for Joint Diseases, New York City.

The tendency has been to assume that hyperparathyroidism is consistently associated with hypercalcemia. We emphasize that frequently this is not the case, that hypercalcemia is not a sole criterion of hyperparathyroidism. Recorded instances of absence of hypercalcemia after parathyroid administration have sometimes been noted as merely paradoxical. The suggestion of "immunity" to parathormone is not supported by the data. On the other hand, the suggestion that an increased rate of excretion may prevent the accumulation of calcium in the blood is plausible. And a generalization (based on work with rodents) that herbivora are very resistant to parathormone injections implies an effect of diet on serum calcium response. However, the resistance of the cat would have to be explained on a different basis.

It has been established that in mice, rats and rabbits relatively small, if any, effects upon serum calcium are produced by administration of relatively huge doses of parathormone. Negative results in guinea pigs were reported by Macleod and Taylor[1] and Taylor.[2]

We found that in guinea pigs consistent effects upon serum calcium and phosphorus could be produced only by very large doses; that these effects could be brought out more prominently, in young animals, after starvation; and that with doses not sufficiently great to produce effects on serum calcium and phosphorus, calcium mobilization and excretion could be demonstrated.

Controls. Normal guinea pigs, young and adult, fed and starved, showed a serum calcium of 10.4±1.0 mg. per 100 cc. The serum phosphorus varied from about 8.0 mg. in guinea pigs weighing

[1] Macleod, J. J. R., and Taylor, N. B., *Trans. Roy. Soc. Can.*, 1925, xix (Sect. V), 27.

[2] Taylor, N. B., *Am. J. Physiol.*, 1926, lxxvi, 221.

about 300 gm. to about 4.0 mg. in adults. Starvation for about 70 hours lowered the serum phosphorus of the young animals to between 5.0 and 6.5 mg.

Effects of a Single Administration of a Large Dose: 1. Calcium. With doses of 10 to 20 units per 100 gm., injected subcutaneously into *adult* guinea pigs, the serum calcium rose to a maximum of about 16.0 mg. about 24 hours after injection and returned within the normal range before 48 hours. Starvation did not intensify the effect. *Young* animals, if fed, showed high serum calcium 24 to 36 hours after injection, and normal values at the 48 hour interval. After about 70 hours' starvation, calcium values were relatively higher at all intervals after injection. Maximum values (15 to 16 mg.) were found between the 18 and 36 hour intervals. High values persisted after 48 hours. Thus, the influence of starvation on the parathormone effect upon serum calcium is shown strikingly not only by the higher values attained but also by their persistence.

2. Phosphorus. The serum phosphorus of young *starved* guinea pigs, after injection of parathormone, rose high beyond the range of values in starved controls—an indication of overdosage, in our opinion. In the *fed* young guinea pigs the effect of parathormone was moderated. In starved *adult* guinea pigs the serum phosphorus was generally raised; the effect was less in the fed adults.

Effects of Repeated Administration of Parathormone. The effect upon serum calcium of single doses of parathormone has been shown to last for more than 24 hours. Daily injections, therefore, result in pyramiding their effect. Six guinea pigs were injected about 7, 14 and 20 units per 100 gm. respectively, on 3 successive days. All of the animals died on the third day. When dosage as large as 30 units per 100 gm. were injected daily for several days, after a previous gradual stepping up from smaller doses, the animals survived until the termination of the experiment. Their serum calcium rose to as high as 20 mg., and the high phosphorus indicated overdosage.

When doses less than 5 units per 100 gm. were injected daily for 10 and 16 days, no clearly recognizable or consistent effects upon the serum calcium or phosphorus were produced. Histological examination, however, showed bone resorption, proving mobilization and excretion of bone calcium in the absence of hypercalcemia.

Thirty-three animals were used as controls, 70 were used for the study of the effects of single injections and 23 for the effects of repeated injections.

Our acknowledgement is due to Eli Lilly and Company for their generous cooperation in supplying most of the parathormone used in these experiments.

4928

Production in Guinea Pigs of Fibrous Bone Lesions with Parathyroid Extract.

HENRY L. JAFFE, AARON BODANSKY AND JOHN E. BLAIR.

From the Laboratory Division, Hospital for Joint Diseases, New York City.

Askanazy[1] found a parathyroid adenoma in a case of *ostitis fibrosa* and he suggested a cause and effect relationship between the parathyroid tumor and the bone disease. After his finding, many other instances of parathyroid enlargement were reported in association with *ostitis fibrosa deformans, ostitis fibrosa cystica,* osteomalacia and rickets. Parathyroid enlargement has also been noted in rats suffering from experimental rickets. The consensus until recently was that the parathyroid enlargement observed in association with these bone diseases was of a secondary nature, and appeared as a result of a compensatory hypertrophy due to the bone deficiency.

Mandl,[2] and after him others, removed parathyroid adenomas in cases of *ostitis fibrosa cystica* and reported rapid clinical improvement of their patients, with cessation of the negative mineral balance.

We felt that, if the extirpation of a parathyroid adenoma resulted in the clinical improvement of a case of *ostitis fibrosa cystica,* injections of parathyroid extract might produce similar or analogous bone lesions, if parathyroid hypersecretion was at the basis of the disease.

Attempts have been made to produce experimental *ostitis fibrosa* by dietary deficiencies and by injury to the bone marrow[3, 4] with negative results.

Dogs treated with parathyroid extract develop very extensive bone resorption, in both the cortex and the medulla, but fibrous repair is difficult to elicit. It is suggestive that in the dog doses large enough to raise serum calcium to very high levels and to produce a recognizable overdosage complex, sometimes leading to death, are not sufficient to cause lesions severe enough to elicit the response of fibrous repair. In the guinea pig, however, doses which, while high in terms of parathormone units, produce very much smaller effects on serum calcium and few, if any, of the overdosage effects, will cause bone lesions with fibrous repair. The reason is probably to be

[1] Askanazy, M., *Arb. a. d. Path. Anat. Inst. Tübingen,* 1904, iv, 398.

[2] Mandl, F., *Arch. f. klin. Chir.,* 1926, xcliii, 1, 245.

[3] Dibbelt, W., *Verhandl. d. deutsch. path. Gesell.,* 1909, xxxiii, 13.

[4] Nissen, R., *Deutsche Z. f. Chir.,* 1925, cxci, 197.

sought in the metabolic differences between the dog and guinea pig, and their differing metabolic responses to parathormone injection. The incidence in man of fibrous dystrophies associated with hyperparathyroidism is probably to be explained by associated or independent metabolic derangements.

In previous attempts to produce *ostitis fibrosa* experimentally, the animals were sometimes kept on deficient diets, as stated above. In our experiments, the guinea pigs were on their normal diet of carrots, cabbage, hay and oats, and most of them gained weight. Sixty-three experimental animals were studied, and fully the same number of controls were also examined.

In the guinea pig we have regularly produced with subcutaneous injections of parathormone the bone changes which satisfy all the criteria of *ostitis fibrosa,* including the appearance of new bone (osteoid). The latter appears as soon as the reparative processes are permitted to operate with sufficient intensity. A guinea pig permitted to go for a few days without parathormone, after previous parathormone treatment, shows an abundance of osteoid tissue beneath the periosteum and endosteum, in the haversian canals, and in fibrous tissue in the metaphysis just distal to the epiphyseal cartilage plate. In passing, we wish to state that the osteoid tissue results not from the metaplasia of the connective tissue, but from rejuvenated osteoblasts present in the scar tissue.

It was of course necessary to use relatively large doses of parathormone to produce fibrous changes in the bones of guinea pigs, compared with the amounts necessary to induce bone resorption in the dog. The resistance of the guinea pig to parathormone has been pointed out,[5] and this resistance is probably attributable to the effect of the exogenous metabolism of the guinea pig, shown by the alkaline urine when fed their normal diet.

We produced bone changes in guinea pigs with as little as 10 units of parathormone, and with 20 to 30 units daily for 3 weeks we obtained very extensive resorption and fibrosis of the bones. Even one dose of 60 units given to a 300 gm. guinea pig produces extensive resorption of the bone, cessation of bone formation and infraction of the cortex in 48 hours. Such guinea pigs may die, with extensive bone destruction, if the large doses are continued. The bone changes described were observed in all the long bones and in the ribs.

Our acknowledgment is due to Eli Lilly and Company for their

[5] Bodansky, A., Blair, J. E., and Jaffe, H. L., PROC. SOC. EXP. BIOL. AND MED., 1930, xxvii, 708.

generous cooperation in supplying most of the parathormone used in these experiments.

4929

The Nerve Pathways in the Vomiting of Peritonitis.

FRANKLIN E. WALTON, ROBERT M. MOORE AND EVARTS A. GRAHAM.

From the Department of Surgery, Washington University School of Medicine, and Barnes Hospital, St. Louis, Missouri.

A careful review of the literature has failed to disclose any explanation of the mechanism of vomiting in peritonitis which is supported by experimental facts. No recorded attempt by critical experiment to determine the nature of the emetic stimulus and to discover its manner of action could be found although the phrase "peritoneal irritation" was noted in practically every work upon peritonitis and vomiting. This present study was undertaken because of the apparent lack of proof that vomiting in peritonitis is really due to irritation of the peritoneum.

Although the emetic stimulus has usually been considered to be a nervous impulse, it was thought possible that it might either be a hormone or a toxin carried in the blood. Since emesis is induced by the direct application to the vomiting center of minute amounts of certain normal constituents of the blood, such as cholin and histamine, it is possible that when one of these is present in the blood in an increased amount, it may cause nausea and vomiting. In intestinal obstruction, which frequently complicates peritonitis, histamine may be present in the blood in abnormally large amounts, and, in such cases, may quite possibly constitute the stimulus to vomiting.

It is believed that observations recently made in this laboratory tend to exclude the probability of either a toxin or a hormone providing the important emetic stimulus in peritonitis. For example, in a series of 15 normal cats intraperitoneal injection of 10 cc. of a 50% turpentine emulsion produced vomiting within 6 seconds after the injection, a reaction time which seemingly would preclude a chemical stimulation of the center and yet be entirely within the limits of a reflex phenomenon. Furthermore, as will be described shortly, peritonitis failed to produce vomiting in 6 cats in which the vagus nerves had been divided in the thorax and the abdominal sympathetic and splanchnic innervation destroyed.

If the emetic stimulus is a nervous impulse, the 3 possible pathways to be considered are the vagi, the sympathetics and those cerebrospinal nerves which also supply the peritoneum, namely the phrenics, lower 6 thoracics, the ilio-hypogastrics and the ilio-inguinal nerves.

The method of study has been as follows: A 24-hour bouillon culture of *B. coli* was injected intraperitoneally into each of 12 normal cats and 7 normal dogs and was found in every instance to produce a fatal peritonitis, the duration of life varying from 5 to 12 days. Without exception, vomiting was a constant feature of the disease. These animals served as controls.

Twelve other cats, which had received similar injections and which also had been subjected to bilateral low intrathoracic vagotomy performed 60-72 hours after injection, failed to differ in any respect from animals whose vagi were intact.

Bilateral abdominal sympathectomy and splanchnotomy was performed in 5 cats. In these animals, a similarly induced *B. coli* peritonitis produced vomiting which did not differ either in character or frequency from that seen in the controls.

In 5 dogs suffering from a colon bacillus peritonitis, the spinal cord was transected at the level of the second thoracic vertebra. Vomiting was feeble in character, thereafter, inasmuch as the innervation of the abdominal muscles was destroyed, but was quite definitely present and persistent.

Six cats were first subjected to a bilateral low intrathoracic vagotomy and allowed to recover. Two weeks later, the abdominal sympathetic chains were resected and the splanchnic nerves divided. After one week of convalescence, an intramuscular injection of lobelein sulphate (0.003 mgm. per kilo) was given and prompt emesis resulted in each instance, demonstrating the integrity of the efferent emetic mechanism. These animals were next given a *B. coli* peritonitis. In this series a striking result was obtained. Every animal died within 6 days' time and postmortem examination revealed in each instance a frank purulent general peritonitis, the diagnosis being confirmed by microscopic section. Yet not one of the 6 animals vomited even once during the course of the disease.

Obviously these animals were able to vomit as demonstrated by lobelein sulphate. The emetic stimulus was present as evidenced by all of the animals previously observed. In these animals then, the emetic action of the peritoneal inflammation had been effectively abolished by the operative procedure, namely by vagotomy and sympathectomy.

In resumè, in each of 46 animals in which either the vagus mechanism or the abdominal sympathetic mechanism or both were intact, vomiting was a prominent accompaniment of peritonitis. In each of 6 animals in which both vagal and sympathetic paths were destroyed, no instance of vomiting was recorded although the peritonitis was uniformly fatal. It would seem then that colon bacillus peritonitis produces vomiting through a local irritation of afferent nerve endings and not through toxic or humoral changes. The afferent emetic impulse from the peritoneum to the medullary centers evidently traverses only vagal or sympathetic paths since in the absence of these paths, although cerebrospinal nerves (including the phrenics) are still active, emesis fails to occur. Since the destruction of only one of these paths, *i. e.*, vagal or sympathetic, fails to abolish the vomiting of peritonitis, it would appear that the afferent emetic impulse traverses either path with equal facility. This observation is in line with that of Hatcher and Weiss,[1] who found that afferent emetic impulses produced by large oral doses of mercuric chloride ascend over either the vagi or sympathetics depending on the integrity of the tract.

Summary. The experiments here reported show that the vomiting of peritonitis is the result of the stimulation of afferent nerve endings located in the peritoneum. The emetic impulse thus initiated passes to the medullary center by way of sensory nerve fibers which are included in both the vagal and sympathetic trunks. Section of these trunks prevents the occurrence of vomiting in peritonitis although phrenic and other cerebrospinal nerve paths are left undisturbed. Since, by sympathectomy alone or by vagotomy alone, vomiting in peritonitis is not abolished, the afferent emetic impulse evidently traverses either path with equal facility.

[1] Hatcher, R. A., and Weiss, Soma, *J. Pharm. and Exp. Therap.*, 1924, xxii, 139.

4930

The Cytochrome of Diphtheria Bacilli and its Relation to Diphtheria Toxin.

C. B. COULTER AND F. M. STONE.

*From the Department of Bacteriology, College of Physicians and Surgeons, Columbia University.**

In the study of substances that may be involved in the oxidation-reduction processes of the living cell attention is drawn to the intracellular pigment cytochrome which was originally observed by McMunn[1] and has recently been rediscovered and renamed by Keilin.[2] This pigment is known only by the characteristic absorption spectrum which is given by the substance in the reduced state; the oxidized form shows no selective absorption or at most a faint shading. Among bacteria this pigment is found in all aerobic forms, and is most distinct in the macroaerophiles. It is absent from the obligate anaerobes. Its occurrence in *B. diphtheriae* has been observed by Yaoi[3] and Tamiya.

We have studied the occurrence of cytochrome in cultures and filtrates of different members of the diphtheria bacillus group. In masses of bacteria sedimented from cultures in broth the characteristic absorption spectrum may readily be seen with the spectroscope if a sufficiently intense beam of light is employed. The absorption bands occupy the wave lengths 613-595, 568-5615, 560-551, and 535-512. If the degree of absorption is great, a single broad band may be seen in place of two in the green, from 568 to 555. The number and position of the bands appear to be the same in the various members of the corynebacterium group. No differences have been seen between toxin-producing and non-toxin-producing strains of the species *B. diphtheriae.*

As with the cytochrome observed by Keilin in various materials, the absorption bands of these bacteria fade out when atmospheric oxygen is admitted to the suspension, and reappear when air is excluded or a strong reducing agent (hydrosulphite) is added.

In the examination of filtrates of the corynebacteria the cultures have been grown in the medium, and according to the procedure,

* This work has been carried out with the aid of a fund established by the Chemical Foundation, Inc., New York City.

1 McMunn, C. A., *Phil. Trans.*, 1886, clxxvii, 267.

2 Keilin, D., *Proc. Roy. Soc., London*, Series B., 1925, xcviii, 312.

3 Yaoi, H., and Tamiya, H., *Sci. Rep. from the Gov't Inst. for Inf. Dis.*, Tokyo, 1927, vi, 85.

employed for the production of diphtheria toxin, after adaptation of the individual strains to growth in a pellicle, After 9 or 10 days of growth the metabolized bouillon was passed through a Berkefeld candle under anaerobic conditions and examined spectroscopically in tubes 200 mm. in length. Observations were made both before and after the addition of a few granules of hydrosulphite to each sample. The results reported here relate to the completely reduced material.

No selective absorption is evident in the sterile unmetabolized bouillon, nor in filtrates from *B. xerosis* and non-toxin-producing strains of *B. diphtheriae.*

Filtrates from toxin-producing strains of *B. diphtheriae* give an absorption spectrum which is well-marked and characteristic and differs in some respects from any absorption spectrum which has been described. A broad band from 555 to 508 with maxima at 536 and 511 corresponds to the absorption in the blue-green by cytochrome and named the "D" band by Keilin. An intense band with maximum at 575 or 576 coincides with one seen in extracts made with strong alkali from yeast by Keilin or by us from the cytochrome-rich *B. phosphorescens.* A band with maximum about 610 corresponds to the "A" band of cytochrome. Another band in the red with maximum between 640 and 630 occupies the position of a band, first observed by Yaoi and Tamiya, in intact cells of *B. dysenteriae* Komagome (but not in Shiga) and in *B. coli.*

Spectrophotometric analyses have shown that a parallel exists between the intensity of spectral absorption and the content of filtrates of various strains in toxin as determined by flocculation tests and animal inoculation. The possibility is, therefore, suggested that diphtheria toxin is derived from cytochrome and that the chemical changes which are involved are similar to those by which a hemochromogen with an absorption at 576 is derived from cytochrome by extraction with alkali.

Just as this work is prepared for publication a note by S. Campbell Smith has appeared in the *Lancet,* March 8, 1930, on the identification of a "porphyrin" in toxic filtrates of *B. diphtheriae.* No relation was observed between the rate of formation of this pigment and the elaboration of toxin. Although the data are meagre, it seems entirely probable that this "porphyrin" is identical with the cytochrome-pigment which we have described.